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ANIMAL POPULATION REGULATION BY THE GENETIC FEED-BACK MECHANISM*

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Natural population regulation has its foundation in the process of evolution. One such process described herein is called the genetic feed-back mechanism. This mechanism through genetic evolution integrates herbivore and plant, parasite and host, and predator and prey in the community. It functions as a feed-back system through the dynamics of density pressure, selective pressure, and genetic changes in the interacting populations. Density influences selection; selection influences genetic make-up; and in turn, genetic make-up influences density. The actions and reactions of the interacting populations in the food chain cycling in this mechanism result in the evolution and regulation of animal populations.

E. B. Ford (1930 and 1931) was the first to point out the importance of genetic changes in population dynamics and specifically as a cause of population fluctuations. He proposed that "numerical increase inevitably prepares the way for reduction, and the reverse; so giving rise to fluctuations in numbers, with alternating periods of high and low variability" (Ford, 1931). During mass increases caused by the changing environment variability increases, and many inferior genetic types result. "These are eliminated, and the numbers reduced when conditions become more rigorous again" (Ford, 1956). A genetic system of population regulation suggested by Franz (1949) proposes that population waves are generated both by inbreeding and the fixation of deleterious genes during mass increases. These are followed by declines initiated by the consequent weakening of the population. The relation between density regulation and natural selection was explored by Haldane (1956), who showed that genetic changes and adaptation for some environmental selective factor may cause a population to increase or decrease to a new stable level. Chitty (1957 and 1960) presented evidence to support the fact that changes in the genetics and viability of field mice are responsible for the population cycles of this species. That "the role of individual differences in population dynamics has been relatively neglected" was emphasized by Wellington (1957), and in a later paper

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(1960) he explained how individual differences might influence populations. Recently, Turner (1960) proposed that population outbreaks follow cross-breeding between isolated inbreeding populations.

From this evidence, it is clear that genetic changes play some role in population regulation, but the manner in which they function is not known. The genetic feed-back mechanism may provide an insight into the nature of this problem.

When a species population enters a new biotic community in which it is not integrated and in which no ecological barrier exists, it reaches outbreak levels a short while after introduction. The following cases amply illustrate this point: Japanese beetle introduced into the United States (Smith and Hadley, 1926), European rabbit introduced into Australia (Stead, 1935), and European gypsy moth introduced into eastern United States (Forbush and Fernald, 1896). The Hessian fly was still another species to reach high density within a short time after its introduction into the United States. In this instance, through genetic change of wheat plants and the consequent alteration of food quality, the density of the herbivore or Hessian fly population was controlled. Previous to 1942, when only susceptible varieties of wheat were grown in Kansas, the Hessian fly population occurred in large numbers across Kansas. When the flies were fed on certain resistant varieties, they suffered high mortality in the larval populations from time of hatching to pupation. If development did take place, growth of the larvae was slow on these resistant varieties (Painter, 1951). When the resistant varieties of wheat were introduced into Kansas in 1942, the Hessian fly population declined in a few years to the point where the flies were too scarce to be used in resistant wheat tests (Painter, 1954). Thus, by changing the genetic make-up of the plant population type this herbivore was feeding upon, the animal population was significantly reduced. If the resistant wheat were exchanged for the original susceptible variety, an increase in the Hessian fly population could be predicted.

The interaction of oysters and a pathogen found in the waters off Prince Edward Island further demonstrates the importance of genetic mechanisms in natural populations. Before 1915, oysters in the Malpeque Bay abounded. During 1915, however, the oyster population was struck by disease. Nearly 90 per cent of the oyster population was infected with the yellowish-green pustules during the first wave (Needler and Logie, 1947). The population of oysters decreased rapidly, and by 1926 no oysters were harvested (figure 1).

Subsequently in 1929, fishermen noticed an increase in oysters, and nearly 500 barrels were removed during 1930. By 1940 production approached the 1915 level of 4000 barrels (figure 1). An investigation to determine if this increase was due to genetic resistance developing in the oyster led Needler and Logie (1947) to import oyster spat from the disease-free Hillsborough River. Over 90 per cent of the Hillsborough spat died of disease whereas the Malpeque Bay spat treated in a similar manner in the bay survived. Malpeque Bay spat were also compared with Enmore River spat. The Enmore River region received the disease about two years previous to this test.

Under the infective conditions at Enmore only 30 per cent of the Enmore River spat survived whereas 98 per cent of the resistant Malpeque Bay spat survived.

Since the pathogen was not isolated, no tests were made of the parasite to determine if any changes in its reproduction and pathogenicity occurred. The oyster itself, however, was well adapted for rapid evolutionary change when it is considered that an oyster produces nearly 60,000,000 young per year (Galtsoff, 1930). This natural event with oysters showed that parasite pressure resulted in genetic change in the oyster-host, and this regulated the density of the parasite itself.

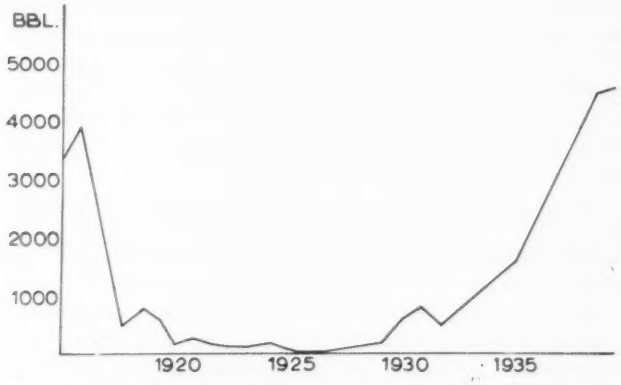

FIGURE 1. Annual yields of oysters from Malpeque Bay and associated regions off Prince Edward Island. (After Needler and Logie, 1947.)

The myxomatosis outbreak in the rabbit population in Australia is another example of the genetic changes functioning in the adjustment of a natural parasite and host population. At Christmas, 1859, the European rabbit (*Oryctolagus cuniculus*) was introduced into Australia and increased rapidly during the next 20 years (Stead, 1935). The extent of the damage caused by these animals prompted the Australian Government to investigate the possibility of introducing some biotic agent which would reduce the density of the rabbit to a less harmful level. The myxomatosis virus obtained from South American rabbits was introduced into the Australian rabbit population. During the first epizootic, myxomatosis was fatal to between 97 and 99 per cent of the rabbits; the second epizootic resulted in 85 to 95 per cent mortality; and the third epizootic resulted in 40 to 60 per cent mortality (Fenner, *et al.*, 1953). The effect on the rabbit population was less severe with each succeeding epizootic, suggesting that the two populations were becoming integrated and adjusted to one another in the ecosystem.

In this adjustment between virus and rabbit, attenuated genetic strains of virus have evolved by mutation and are tending to replace the virulent strains (Thompson, 1954). In addition, passive immunity to myxomatosis is

conferred to kittens born of immune does (Fenner, 1953). Finally, a genetic change has occurred in the rabbit population, and this is providing intrinsic resistance to myxomatosis (Marshall, 1958). Here definitely is illustrated the functioning of the feed-back of density, selection, and genetic change which has in turn altered the density of both populations. In this particular association, genetic change seems to be taking place in both populations. The virulent genetic strain of virus has an apparent advantage in that it reproduces quickly and can rapidly convert rabbit protoplasm into itself. This is an advantage only when this virus is compared to another which has a slower rate of increase within the rabbit. The virus strains, however, must be evaluated in the ecosystem to determine which strain ultimately has the adaptive advantage. As Li (1955) points out, "adaptability is a response of populations rather than of the individual." If the virulent strain were to become 100 per cent effective in attacking the rabbit in the ecosystem, it would destroy itself by destroying its food supply. A less virulent strain in this case would tend to have greater survival value when the strains are separated diversely in space. The non-pathogenic strain observed in Australia has a selective advantage in this region.

Transmission of the myxomatosis virus depends upon mosquitoes (Aedes and Anopheles) which feed only on living animals (Day, 1955). Rabbits infected with the virulent strain of virus live for a shorter period of time than those infected with the less virulent strain. Because rabbits infected with the less virulent strain live for longer periods of time, mosquitoes have access to that virus for longer periods of time. This provides the non-virulent strain a competitive advantage over the virulent strain. In addition, in regions where the non-virulent strain is located, rabbits are more abundant, and this allows more total virus to be present than in a comparable region infected with the virulent virus. Thus, the virus with the greatest rate of increase and density within the rabbit is not the virus selected for, but it is the virus whose demands are balanced against supply which has survival value in the ecosystem.

The above mentioned natural populations were regulated by genetic changes operating in the feed-back mechanism. The interactions and trends which operated in this mechanism are translated into biomathematics and explained in the following model using a herbivore-plant system. This proposed feed-back mechanism functions through polygenic action, but to illustrate the process two alleles (A, a) at one locus are adequate. The alleles in various proportions ($p^2 + 2pq + q^2 = 1$) in the diploid plant determine whether an animal population feeding on the plant increases or decreases, where p^2 is the proportion of homozygous dominants (AA), $2pq$ is the proportion of heterozygotes (Aa), and q^2 is the proportion of homozygous recessives (aa) in the plant population. The animals distribute themselves equally on the individual plants, and animal reproduction (R) varies according to the plant genotype on which they are feeding. For example, on the AA plant animal reproduction (R_p) is 2, on the Aa plant animal reproduction (R_{pq}) is 1, and on the aa plant animal reproduction (R_q) is 0.5. Thus animal

density (N) at an initial time (N_{to}) increases or decreases in the following generation (N_{t1}) depending on the proportion of the AA, Aa, and aa plant genotypes. In this case then

$$N_{t1} = p^2R_pN_{to} + 2pqR_{pq}N_{to} + q^2R_qN_{to}. \tag{1}$$

Environmental selective pressure (S), or survival (1 – S), and animal density pressure determines the genotypic proportions of the plant population at the next generation. For this example, environmental selective pressure on the AA plant (S_p) is 0.001, on the Aa plant (S_{pq}) is 0.2, and on the aa plant (S_q) is 0.6. In a situation without the animal the "relative fitness" (Li, 1955) of the genotypes is such that the AA plants are better than the Aa plants, and the Aa plants are better than the aa plants ($p^2 > 2pq > q^2$). Plant tolerance for animal density varies according to plant genotype. At one maximal animal density (b) none of the AA plants survive; at another density (c) none of the Aa plants survive; and still at another density (d) none of the aa plants survive. Pressure by the animal on the AA, Aa, and aa plant genotypes is proportional to animal density as $\frac{b-N}{b}$, $\frac{c-N}{c}$, and $\frac{d-N}{d}$, respectively. Thus the selective pressure against the plant genotypes exerted by animal density and the other environmental factors in the ecosystem is

$$p^2(1-S_p)\frac{(b-N)}{b} + 2pq(1-S_{pq})\frac{(c-N)}{c} + q^2(1-S_q)\frac{(d-N)}{d} = G. \tag{2}$$

The total proportion of the plant population surviving is G. Following the selection, the proportions of the surviving plant genotypes are

$$\frac{p^2(1-S_p)\frac{(b-N)}{b}}{G} + \frac{2pq(1-S_{pq})\frac{(c-N)}{c}}{G} + \frac{q^2(1-S_q)\frac{(d-N)}{d}}{G} = 1. \tag{3}$$

The proportion of the A allele (p) surviving is $p^2 + \frac{1}{2}(2pq) = p$ and the proportion of the surviving a allele (q) is $q^2 + \frac{1}{2}(2pq) = q$. By random mating the surviving allelic proportions,

$$(p+q)^2 = 1. \tag{4}$$

The proportions of the plant genotypes present in the next generation are

$$p^2 + 2pq + q^2 = 1. \tag{5}$$

For the present example, the maximum animal density (b) for survival of the AA plants is 1075; the maximal animal density (c) for the Aa plants is 1500; and the maximal density for survival of the aa plants is 3000. Logically the genotype destroyed by the lowest maximum animal density is the genotype on which the animal has the highest reproduction rate. If we now imagine 150 animals being introduced into the ecosystem and the proportions of the plant genotypes are .360 AA plants, .500 Aa plants, and .140 aa

plants and the numerical example is fed into a digital computer and cycled through equations 1, 2, 3, 4, and 5 for 100 generations, we find that the animal population fluctuations decrease in amplitude with each generation and stability is reached at an animal density of 795 in 72 generations (figure 2). The genotypic plant proportions at stability are .172 AA plants, .485 Aa plants, and .343 aa plants.

The model was revised and made more complex to allow the genetics of the animal population to vary in addition to density. Changes in the plant population remained similar to those in the previous model. The relation of animal genetics in this system is represented by two alleles (Z, z) at one locus in the animal. The proportions of ZZ are indicated by x^2, of Zz by $2xy$, and zz by y^2. It is assumed that the three animal genotypes (ZZ, Zz, zz) reproduce differently on each of the three plant genotypes (AA, Aa, aa).

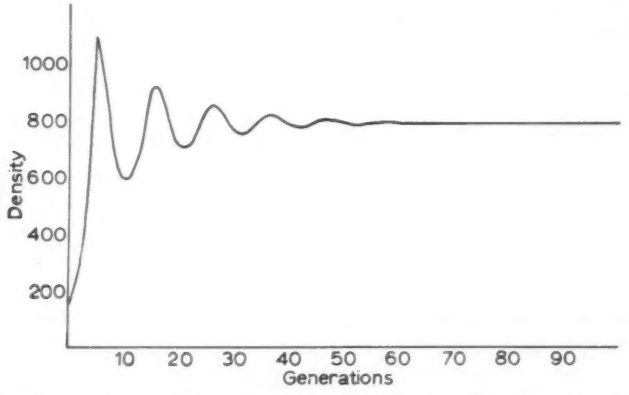

FIGURE 2. Animal population regulation resulting from the interaction of plants and animals in the genetic feed-back mechanism. Starting conditions: p^2 (.360), $2pq$ (.500), q^2 (.140), N_{to} (150), S_p (.001), S_{pq} (.2), S_q (.6), b (1075), c (1500), d (3000), R_p (2), R_{pq} (1), 2nd R_q (.5).

Hence, on the AA plants the ZZ animals reproduce I_x, the Zz animals reproduce I_{xy}, and the zz animals reproduce I_y. On the Aa plants, the ZZ animals reproduce J_x, the Zz animals reproduce J_{xy}, and the zz animals reproduce J_y; and on the aa plants the ZZ animals reproduce L_x, the Zz animals reproduce L_{xy}, and the zz animals reproduce L_y. Reproduction of the animal and survival of the animal types depends on the proportion of the plant genotypes. Animal reproduction, of course, on the various plant genotypes depends on the proportion of the animal genotypes. Thus the proportion of the animal population on the AA plants produce the following animal numbers

$$N_{t1p} = N_{to}\,p^2x^2I_x + N_{to}\,p^2 2xyI_{xy} + N_{to}p^2y^2\,I_y$$

and on the Aa plants the animals produce

$$N_{t1pq} = N_{to}\,2pqx^2J_x + N_{to}\,4pqxyJ_{xy} + N_{to}\,2pqy^2J_y$$

and on the aa plants the animals produce

$$N_{toq} = N_{to}\,q^2x^2L_x + N_{to}\,q^2\,2xyL_{xy} + N_{to}\,q^2y^2L_y\,.$$

Animal density for the following generation is

$$N_{t1} = N_{t1p} + N_{t1pq} + N_{t1q}\,. \tag{6}$$

The proportions of the surviving animal genotypes x^2, $2xy$, and y^2 respectively are

$$\frac{N_{to}\,p^2x^2I_x + N_{to}\,2pqx^2J_x + N_{to}\,q^2x^2L_x}{N_{t1}}$$

$$+\frac{N_{to}\,p^2\,2xyI_{xy} + N_{to}\,4pqxyJ_{xy} + N_{to}\,q^2\,2xyL_{xy}}{N_{t1}} \tag{7}$$

$$+\frac{N_{to}\,p^2y^2I_y + N_{to}\,2pqy^2J_y + N_{to}\,q^2y^2L_y}{N_{t1}} = 1\,.$$

The proportion of the Z allele (x) of the animals surviving is $x^2 + \frac{1}{2}(2xy) = x$ and the proportion of the surviving z allele (y) is $y^2 + \frac{1}{2}(2xy) = y$. By random mating the surviving allelic proportions of the animal,

$$(x + y)^2 = 1 \tag{8}$$

the proportions of the animal genotypes in the next generation are

$$x^2 + 2xy + y^2 = 1\,. \tag{9}$$

Then with the animal density (N_{t1}) we pass through equations 2, 3, 4, and 5 as in the first model. Starting with the conditions listed in figure 3 and cycling these data through equations 6, 7, 8, 9, 2, 3, 4, and 5, population cycles result for 2100 generations. Only 100 generations are graphed in figure 3. During the 2100 generations the amplitude of the cycles decreased by only four individuals. By extrapolation, the population would continue to cycle for about 20,000 generations.

Starting with conditions similar to those listed in figure 3, except that $S_{pq} = 0.2$ and $S_q = 0.6$, the population stabilizes in 100 generations, and there results a population trend similar to that graphed in figure 2.

Numerous other models were constructed. In one the animal passed through ten generations while in the plant there was one generation; in another, animal reproduction was made density-dependent, and in another, plant density varied in addition to its genetic changes. The feed-back mechanism regulated in all cases.

A longer time is required for the model to stabilize when the starting animal density is relatively far from the stable value, when the starting genotypic proportions are far from the stable values, and if the differences in either the environmental selective pressure or the maximum animal densities

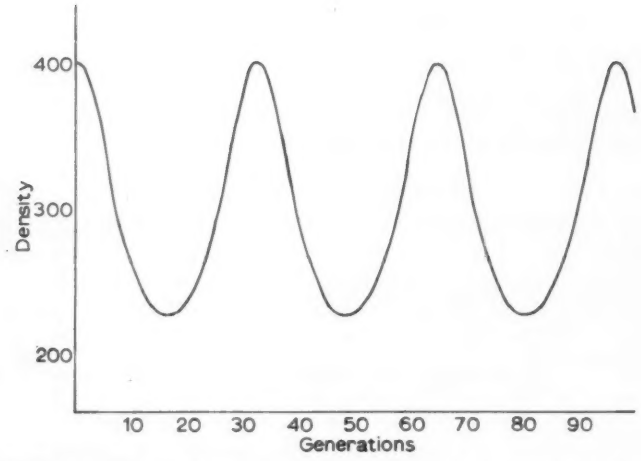

FIGURE 3. Animal population cycles resulting from the interaction of plants and animals in the genetic feed-back mechanism. Starting conditions: p^2 (.172), 2pq (.485), q^2 (.343), x^2 (.250), 2xy (.500), y^2 (.250), N_{to} (400), S_p (.001), S_{pq} (.1), S_q (.2), b(1075), c (1500), d (3000), I_x (2.8), I_{xy} (2.2), I_y (.8), J_x (1), J_{xy} (1), J_y (1), L_x (.1), L_{xy} (.4), and L_y (1.1).

for the survival are small between each plant genotype. For example, using 0.001, 0.100, and 0.200 for environmental selection pressures for the respective genotypes, AA, Aa, and aa, has greater instability than larger differences such as 0.001, 0.200, and 0.600, respectively. Similarly the maximum densities, 1000, 2000, and 3000, contribute less stability than larger differences such as 1000, 3000, and 9000. Any change occurring in the environmental pressure, in the animal reproduction on the plant genotypes, or in the maximum animal density for the survival of each genotype will change the stabilizing density of the animal to a new level or change the stable values for both the animal density and the plant genotypic proportions.

The feed-back mechanism will regulate under any number of combinations of starting conditions if (1) animal reproduction on the AA plant is greater than that on the aa plant, (2) the animal reproduces greater than one on any one of the genotypes and less than one on another, and (3) the AA plants have a greater survival value than the aa plants in the ecosystem without the animal. Conditions with the homozygous plant genotypes, of course, can be reversed.

The biomathematical models allowed simplification and thus a better understanding of the functioning and action of the feed-back mechanism. In nature the feed-back mechanism would be based most likely on a multifactorial system instead of the unifactorial genetic system used in the models. A multifactorial system would have greater flexibility, and, of course, greater complexity. Obviously the evolution of population regulation in nature would be most difficult to follow because of the nearly infinite number

of changing environmental factors. The addition of these other factors will not change the course of evolution in population regulation, but these factors will make the course devious.

With the biomathematics as a base of understanding the function of the mechanism, further details are needed to relate it to biological systems. In all cases, the density of the herbivore populations is a function of both birth rate and death rate, excluding dispersal. Both birth rate and death rate of the herbivore are a function of the quality of food provided by the plant. The food quality is a function of the genetic characteristics of the plant population which in turn are a function of the selective pressure exerted on the plant by the herbivore. Therefore, herbivore density at an initial time determines herbivore density at some future time. So the cycle continues with the density-dependent mechanism regulating the size of the herbivore population.

It is natural that animal reproduction on the AA plants in the models is greater than that on the aa plants and at the same time the AA plants possess a greater survival value than the aa plants in the ecosystem without the animal. If the animal-resistant aa plant possessed greater survival value than the animal-susceptible AA plants, then the animal could not have established itself because of this initial ecological barrier. Whenever the animal is absent or at low density, the plant population gradually shifts until there exists a higher proportion of AA plants than aa plants. In the ecosystem without the animal, the AA plant has an advantage because it has greater survival value than the aa plant. In contrast, when animal density is high, the aa plant has greater advantage than the AA plant because it is more resistant to animal attack.

Man's selection, which operates to produce plants and animals adapted to artificial environments, has sacrificed characters which are necessary to the animals if they are to compete and survive with natural forms in the wild (Srb and Owen, 1953). Because the resistant genes which would allow a plant or animal to survive in an ecosystem that now includes some attacking animal were existing at low frequency in the original ecosystem, it must be assumed that these resistant genes at high frequency are to some extent disadvantageous—if this were not so, then the genes would have been common in the population in the original ecosystem (Crow, 1957).

Organisms only maintain characters important to their survival. Degeneration of skin pigmentation and eyes in cave fishes results from decreased selection for these characters and increased selection for sensory barbels and other characters which lead to better adaptation to the cave environment (Hubbs, 1938). An organism adapting itself to some special feature of the environment, like resistance to animal attack, must sacrifice certain organs or functions for the greater efficiency of others (Huxley, 1943). This efficiency is basic to the economy of the organism itself. Because the dominant selective pressures receive special attention, they tax the other systems of the organism for support.

Then with relaxation of the new selective pressure, a reversion to original genetic type will result, following the principle of "genetic homeostasis" (Lerner, 1954). Darwin (1859) was aware that domesticated animals if not under continuous selection tended to revert to original wild stocks. Examples of reversion following the withdrawal of selective pressures on laboratory populations are numerous. Mather and Harrison (1949) and Streams (1960) found that Drosophila selected for high bristle number tended to return to their original low bristle number when selection ceased. Resistance to insecticides by house flies was lost when this selective pressure was removed from the populations (Pimentel *et al.*, 1953; Varzandeh *et al.*, 1954; Barbesgaard and Keiding, 1955). Populations of microorganisms lost their high level of resistance to drugs when cultured under normal conditions without the drug (Cardot and Laugier, 1923; Morgenroth, 1924; Dettwiler and Schmidt, 1940; Schmidt *et al.*, 1942; Davies and Hinshelwood, 1943; Fulton and Yorke, 1943).

The genetic change in the plant necessary to reduce the herbivore population itself is determined by the nature of the environment. When environmental pressure is severe then only slight genetic change is needed in the plant to reduce the herbivore population. However, when the environment is highly favorable for the herbivore, the pressure on the plants becomes more intense, and the change in the plant has to be significant to reduce the herbivore population to a point of balanced supply and demand.

The rabbit-virus model mentioned earlier presents evidence concerning the question of differential rates of evolution between the eaten and eating species—this is also true of the interactions between cynipids and their oak-tree hosts. Because cynipids pass through more generations than oak trees per unit time, a faster rate of evolutionary change in the cynipids is possible than in oak trees. Similarly, the myxomatosis virus has a greater capacity for change in a short period when compared with rabbits. Natural selection does not favor the cynipids or virus which evolves and destroys its host, but favors the cynipids or virus which allows survival of its host. Through individuals and colonies selection favors the balanced supply-demand system. The evolution of the virus-rabbit association substantiates this fact.

In some cases where animal pressure is severe and the animal must contend with competitive and other environmental factors, the plant may evolve faster than the animal. This, however, is not a requisite for the successful operation of the system. Evolution in each animal is toward survival and the leaving of reproductively successful progeny. Beyond a certain point, the animal gains no advantage in overcoming the plant's resistance to leave more progeny. Selection does not favor a high reproductive rate per se but a reproductive rate profitable from the standpoint of producing successful progeny. Fair apportionment of nutriment must be made between that devoted to maintenance of the parent and production of young (Fisher, 1930) and in some cases maintenance of these young upon birth. Therefore, any

increase in fertility is made at the expense of other survival traits (Smith, 1954; Cole, 1957). Selection for lower reproductive rates has been documented by Lack (1956). He found that in "Switzerland, the alpine swift (*Apus melba*) normally lays three eggs"; the number of young which fly from broods initially consisting of one, two, three, and four young was respectively, 1.0, 1.7, 2.4, and 2.2. Thus, the largest brood (four) produced fewer successful young than the smaller brood of three.

Selection for the most productive birth rates, not necessarily the highest, occurs in family units (Birch, 1960; Emerson, 1960), colonies (Emerson, 1958), and individuals (Root, 1960). Fundamental to optimal reproduction is the supply and demand economy which exists between individuals or colonies of the herbivores and plants, parasites and hosts, and predators and prey. On partially isolated trees an insect colony which evolves to reproduce at a high rate by converting the trees into insect protoplasm at a rapid rate may destroy the trees and in the end produce fewer successful young than another colony with a lower reproductive rate (Wright, 1960). Viruses and bacteria, which attack relatively long-lived plants and animals, do not evolve rapidly to high reproductive types and greater pathogenicity. Thus, the drift of evolution is toward optimal reproductive types which will provide a balanced economy of supply and demand between the eating and eaten species.

Population birth rate of the animal is influenced either by alterations in the quality of the food or alterations in the intrinsic nature of the eating individuals themselves. Beadle (1945) has experimentally developed genetic strains of Neurosopora which were unable or inefficiently able to synthesize various necessary vitamins and amino acids from a minimal medium. Some of the genetic strains were unable to grow while others grew at reduced rates on such medium. Thus, by altering the genetic make-up of the individual and correspondingly the biochemical structure of the organism, its functional rate of growth can be changed, and, of course, this will influence the dynamics of such a population.

The feed-back mechanism is in accord with the fact that the majority of animal species are rare in nature (Darwin, 1859; Andrewartha and Birch, 1954; Milne, 1960). The herbivore, parasite, or predator population cannot be abundant compared with its respective plant, host, or prey population. In the balanced economy, the eating species feed on only the interest (excess individuals of the eaten population) and do not touch the capital (those individuals necessary for the maintenance of the population of the eaten species).

That the genetic feed-back mechanism functions as a regulatory system in herbivore-plant and parasite-host relationships is supported by evidence from the biomathematics of population dynamics and studies of natural populations. The principles of the mechanism also apply to predator-prey systems. The importance of the feed-back mechanism as a regulatory system is substantiated by its wide application to such diverse interacting sys-

tems; however, the significance of the mechanism lies in the fact that it has its foundation in evolution. The mechanism follows Dr. A. E. Emerson's view that evolution is toward increased homeostasis within populations and the ecosystem (Emerson, 1960).

The task which lies ahead is to determine how the feed-back mechanism functions in regulating natural populations and what its relationship is to the other regulatory theories like "competition" (Nicholson, 1933; Varley, 1947; Solomon, 1949; DeBach, 1958; Morris, 1959; Holling, 1959; Watt, 1959), and "environmental randomness" (Thompson, 1929; Andrewartha and Birch, 1954; Milne, 1957). I do not propose that the feed-back mechanism is the only means of population regulation, nor that this mechanism is independent of the "competition" and "environmental randomness" ideas. The three are interdependent, and I suspect that upon the introduction of a new animal type into a new ecosystem there is an evolution of regulation from both the "competition" and "environmental randomness" conditions to the feed-back mechanism. That is, before sufficient change takes place in the eating population and eaten population, the principal means of regulation is through "competition" and "environmental randomness."

In well-designed experiments lie the challenge and necessary evidence concerning the various control mechanisms. The validity of the feed-back mechanism is under experimental investigation both in the laboratory and field by myself and students of ecology at Cornell University.

I wish to acknowledge the stimulating and pleasant discussion in person and through the mails with Drs. H. G. Andrewartha, W. L. Brown, L. C. Cole, A. E. Emerson, J. Franz, A. Milne, A. J. Nicholson, T. Park, and B. Wallace and Mr. F. R. Streams. Their arguments have helped me clarify my own concept of this mechanism. These discussions have underscored the need for research, and as Claude Bernard (1865) emphasized, "we shall reach really fruitful and luminous generalizations about vital phenomena only insofar as we ourselves experiment and, in hospitals, amphitheaters, or laboratories, stir the fetid or throbbing ground of life."

SUMMARY

That a genetic feed-back mechanism functions to regulate populations of herbivores, parasites, and predators is supported by evidence from the biomathematics of population dynamics and studies of natural populations. The mechanism functions as a feed-back system through the dynamics of density pressure, selective pressure, and genetic changes in interacting populations. In a herbivore-plant system, animal density influences selective pressure on plants; this selection influences genetic make-up of plant; and in turn, the genetic make-up of plant influences animal density. The actions and reactions of interacting populations in the food chain cycling in the genetic feed-back mechanism result in the evolution and regulation of animal populations.

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RARE PARTHENOGENESIS IN *DROSOPHILA ROBUSTA*

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Unmated females of certain animals, when held under domestication or in the laboratory, will produce large quantities of unfertilized eggs. This situation has made possible the discovery of an exceedingly low rate of natural parthenogenesis in forms which have previously been thought to reproduce wholly by sexual means (for example, Stalker, 1954, for various Drosophilidae; Yao and Olsen, 1955, for turkeys; Laven, 1957; Kitzmiller, 1959, for mosquitoes). Except for *D. parthenogenetica* (Stalker, 1954) and *D. mangabeirai* (Carson *et al.*, 1957), unmated Drosophila females of various species produce adult impaternate offspring only very rarely. Thus, Stalker screened somewhat less than 1,000,000 unfertilized eggs of 29 such species and obtained only three adults, two of *Drosophila polymorpha* and one of *Drosophila affinis*. The present account gives the results of extensive screenings of unfertilized eggs of *Drosophila robusta*.

MATERIAL AND METHODS

Over a period of three years, the writer performed an extensive series of selection experiments on *Drosophila robusta* (Carson, 1958). The technique calls for the isolation of females in groups of 50 of close to the same age. These flies are held on well-yeasted food for one week after which two females of the group of 50 were selected as parents and the remainder discarded. Approximately 13,800 females were handled in this way. Beginning in October, 1955, however, instead of discarding females eliminated by the selection procedure and their controls, the remaining flies were placed in a fresh bottle and retained. Each group of females was kept thereafter for four weeks; during this time each group of flies had a sojourn of one week in each of four culture bottles. Also, during this time ample fresh yeast was available to the flies and oviposition was continuous. After the females were removed from a bottle, a few males were added; these served to keep down growth of bacteria and moulds. After a week, these males were discarded. The bottles were then set aside and examined to see if any adult flies emerged. No bottle was discarded as negative until the youngest eggs therein were 21 days old. The time for development from egg to adult for *D. robusta* is 15–16 days at 25°C. which was the temperature maintained during these experiments.

The number of unfertilized eggs laid per bottle was not counted but has been roughly estimated in the following way. Unpublished data of the writer and Dr. H. D. Stalker indicate that a single unmated female of *D. robusta* under optimal conditions will lay from 67 to 91 eggs per day from the sixth to the 35th day of age. Accordingly, it is felt that under conditions of

moderate crowding, the number of eggs per female per day will be considerably less; 25/day (175/week) is taken as a moderate estimate. The average number of females per bottle was taken at approximately 30; each group started with 45 or 50 but dwindled during the four weeks due to deaths. Thus, 30 females laying 175 eggs per week in a bottle would produce 5250 eggs per bottle per week; in round numbers, then, each bottle may be said to contain about 5000 eggs. This is probably an underestimate, but the order of the magnitude is believed to be correct. Twelve strains were studied; each strain originated from a single pair of wild flies collected in nature. Ten of the strains originated from flies collected at Chadron, Nebraska (C6, C15, C39, C66, C72, C77, C79, C95, C102, C109), and two from flies collected at Steelville, Missouri (Sv135, Sv136). Further information on these strains will be found in Carson (1958).

Bottles were examined several times a week for the emergence of an adult fly. When such a fly was found, the following procedure was generally carried out. The fly was isolated, etherized and examined for sex and any morphological peculiarities. If it was reasonably freshly emerged, this fact could be recorded because it takes several days for a specimen of *D. robusta* to attain the dark body color characteristic of the adult. The food mass was systematically searched until the empty pupal case from which the fly emerged was found. The approximate time of development from egg to adult for such a fly was also calculated.

RESULTS

Between January, 1956, and March, 1957, a total of 14 parthenogenetically produced flies were found. A total of approximately 10,585,000 eggs was screened during this period, giving an overall rate of more than one adult female per million eggs (1.32×10^{-6}). Figure 1 shows diagrammatically the origin of each female. The open circles, containing strain numbers, represent a large number of unmated females which were tested from each original strain. Below the symbol is given the approximate number of unfertilized eggs screened. The solid circles represent impaternate females, and are numbered 1–14.

From the 12 original strains, seven (Nos. 1–7, figure 1) impaternate females were obtained from about 7,500,000 eggs, a rate of approximately 0.93 flies per million eggs. Among the offspring of flies having a history of parthenogenesis in at least one ancestor, there were seven females (Nos. 8–14) obtained from approximately 3,085,000 eggs or a rate of 2.23 flies per million eggs. Each of the original seven flies came from a different strain. In most cases they were not held long as virgins but were crossed to males and their female progeny tested again in large numbers, if possible, for parthenogenesis. Notes on the characteristics and fertility of these females will be found in table 1. Only three of the seven flies from the original stocks appeared to have full fertility (Nos. 1, 4, and 7). Nos. 5 and 2 produced only female offspring. Parthenogenetic females 8–14 were pro-

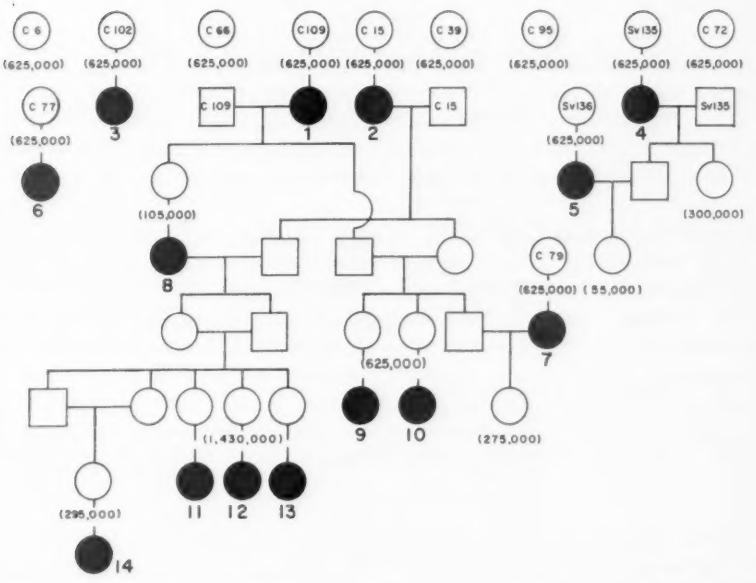

FIGURE 1. Pedigrees of impaternate females (Nos. 1–14) of *Drosophila robusta* (solid circles). The open circles in most cases represent sibships of unmated females; below these symbols is given the number of unfertilized eggs screened in tests for parthenogenesis. Each strain is numbered: (C = Chadron, Nebraska; Sv = Steelville, Missouri).

duced in lines which were descended from Nos. 1 and 2. Of these, only No. 8 gave offspring (figure 1, table 1).

DISCUSSION

In all likelihood, the parthenogenetic females found in this study arise through automictic fusion of meiotic nuclei in the cytoplasm of the unfertilized egg (see Stalker, 1954, 1956; Carson *et al.*, 1957; Murdy and Carson, 1959; Metz, 1959; Sprackling, 1960). It is not possible to say, however, whether aneuploidy or polyploidy is involved because in only one case was the diploid chromosome complement actually observed in an impaternate female. There seems little question, however, that the genetic state accompanying parthenogenesis is an unbalanced one; this is manifested by the rather considerable degree of sterility encountered among these females.

Capacity for rare parthenogenetic development was demonstrated to exist in seven different wild strains out of 12 studied; five of these strains originated from a single Nebraska locality and two came from a single Missouri locality. Such a finding does not preclude the possibility of the existence of strains with much higher rates, but nevertheless it is clear that the present finding is not due to a single, locally peculiar genetic situation.

TABLE 1

Fertility and other characteristics of impaternate females of *Drosophila robusta*

Female No.	Source of males used as mates	Fertility	Morphological abnormalities	Remarks
1	C109	Fertile; produced many offspring of both sexes	None	Laid eggs but produced no larvae or brown eggs as a virgin
2	C15	Partially fertile; produced only 2 offspring, both females	Slightly abnormal abdominal sternites	Became "plugged"; had large ovaries; had normal 2n chromosome group
3	C102	Sterile	Abnormal abdominal sternites	Laid eggs but produced no larvae or brown eggs as a virgin
4	Sv135	Fertile; produced many offspring of both sexes	None	
5	F_1 from No. 4	Partially fertile; produced 68 offspring, all females	None	Held in cool temperature prior to mating
6	C77	Sterile	None	
7	See fig. 1	Fertile; produced many offspring of both sexes	None	
8	See fig. 1	Partially fertile; several hundred offspring of both sexes	One deformed wing and abnormal abdominal sternites	
9	F_1 from No. 8	Sterile	None	
10	F_1 from No. 8	Sterile	None	
11			None	Not mated; held on food; died after 7 weeks
12			Irregular abdominal sternites	Not mated; died after 4 weeks
13			Grossly abnormal abdominal sternites and tergites	Not mated; died after 2 weeks
14	C66 stock	Sterile	Contained no ovaries	Lived 6 weeks with males

Calculation of rates must be done with caution because of the crude way in which the number of eggs has been estimated. Nevertheless, the method of estimation is at least internally consistent in these studies. The rate of parthenogenesis in the initial 12 strains has been calculated as .93 adult females per million eggs. This appears not to be inconsistent with the results of Stalker (1954) who found two dead embryos (but no adults) in approximately 11,000 eggs of *D. robusta* examined. The second seven parthenogenetic females were all found in lines descended from two of the original

ones (Nos. 1 and 2). The rate found in eggs laid by virgin females in whose ancestry is one or more parthenogenetic females is 2.2 adult females per million eggs, or about 2½ times the rate found in the original strains. Such an increase under such selection indicates that the parthenogenesis has a genetic basis and is not unexpected in view of the striking increases in rates obtained by Stalker (1954) by selection in *D. parthenogenetica* and *D. polymorpha*.

The existence of low rates of genetically-based parthenogenesis in many species of Drosophila is of particular interest in that a clear case has recently been found wherein the species concerned, *Drosophila mangabeirai*, reproduces wholly by obligatory thelytokous parthenogenesis (Carson *et al.*, 1957; Carson, 1961). Whereas there is no evidence that *D. parthenogenetica*, *D. polymorpha*, or *D. robusta* have any tendency to depend on parthenogenesis in natural populations, *D. mangabeirai* clearly has reached this stage in the evolution of parthenogenesis. Males are unknown in the laboratory and are apparently produced only as great rarities in nature.

SUMMARY

1. By screening large quantities of unfertilized eggs laid by unmated females in the laboratory, 14 parthenogenetically-produced individuals of *Drosophila robusta* were found. All were females. It is suggested that they arose through automictic fusion of haploid products of meiosis in the unfertilized egg.

2. Each of the initial seven females came from a different wild strain, five from Nebraska and two from Missouri. The rate of parthenogenesis in these strains was .93 adult females per million unfertilized eggs laid.

3. Virgin females with one or more parthenogenetic females in their ancestry show a rate of parthenogenesis about 2½ times that of the original strains.

4. A majority of the parthenogenetic females show reduced fertility, complete sterility and/or morphological abnormalities.

5. The evidence indicates that rare parthenogenesis such as is found in *D. robusta*, *D. parthenogenetica*, and *D. polymorpha* is genetically based and may serve as an example of an evolutionary stage through which the obligatory parthenogenetic species, *D. mangabeirai*, may have passed during the evolution of its parthenogenesis.

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MEIOTIC DRIVE IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*. VI. A PRELIMINARY REPORT ON THE PRESENCE OF SEGREGATION-DISTORTION IN A BAJA CALIFORNIA POPULATION*†

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Meiotic drive is a term coined by Sandler and Novitski (1957) to describe the situation whereby a heterozygote produces gametes containing an excess of one allele, rather than the expected equality. As a consequence of such aberrant segregations, gene frequencies within a population may be radically altered; indeed, detrimental or lethal genes closely linked to the driven gene may increase in frequency, thereby leading to a reduction in fitness or possibly even to extinction of the population.

An instance of meiotic drive in *Drosophila melanogaster*, discovered by Hiraizumi in a Madison, Wisconsin, population, has been reported by Sandler, Hiraizumi and Sandler (1959). The phenomenon, termed *segregation-distortion*, was found to depend upon a locus designated *SD*, which is located in the centromeric heterochromatin of chromosome II. The phenomenon is expressed in males only.

Since segregation-distortion was originally found in a natural population, the question arose as to whether the phenomenon is of recent origin and therefore localized in the vicinity of Madison, or well established and hence widespread among natural populations of *Drosophila melanogaster*. Therefore, the screening of wild populations for *SD* (or for any other type of aberrant segregation) was undertaken. In small samples from only thirteen populations, *SD* has been detected in one Baja California population as well as in three other rather widely separated Madison populations. This report will be confined to establishing that the locus discovered in the southern Cape region of Baja California (specifically, from Rancho La Burrera near the west base of Sierra de La Laguna, and a Pleistocene lake relic, called La Laguna, in the same range) is indeed *SD*.

In testing the abnormal Baja California chromosome for identity with *SD*-bearing chromosomes from Madison, the following criteria must be satisfied: (1) A male heterozygous for *SD* and a structurally normal SD^+ chromosome (marked with the recessive eye-color mutants *cn* and *bw*) testcrossed to *cn bw* females produces progeny among which almost all, rather than the expected 50 per cent, possess the *SD* chromosome. (Since no crossing over occurs in male Drosophila, *SD* will never recombine with the mutant markers.)

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(2) The progeny of a heterozygous female, however, show a normal 1:1 segregation ratio; but her heterozygous sons, when testcrossed, will again exhibit an abnormal *k* value unless conditional distortion has occurred (Sandler and Hiraizumi, 1959). The *k* value is defined as the proportion of *SD*-bearing sperm. From an ordinary heterozygote it is expected to be 0.50; *SD* heterozygotes, however, usually show *k* values between 0.90 and 1.00. (3) When the *SD* chromosome is made heterozygous with inversions having one breakpoint near the centromere, such as *In(2LR)Cy*, segregation-distortion is inhibited; that is, *SD/Cy* males give only 50 per cent *SD* in the progeny. (4) An *SD* chromosome is not sensitive to the distorting action of another *SD* chromosome; that is, homozygous *SD* exhibits normal 1:1 segregation. (5) The locus of *SD* lies in the centromere region, between the markers *pr* and *cn*.

The original six *SD* chromosomes recovered in Madison fell into two categories: *SD*-5 type, carrying a lethal gene associated with a proximal inversion plus a large distal inversion which together very effectively suppress crossing-over on the entire left arm; and *SD*-72 type, lethal free and bearing only the distal inversion. The latter type has possibly been derived from the former by loss of the small proximal inversion and the associated lethal effect (Hiraizumi, Sandler and Crow, 1960). Absence of a lethal gene in line *SD*-72 indicates that its presence is not necessary for the *SD* effect. All of the Baja California chromosomes tested were lethal free.

Furthermore, recovery of recombinants expressing *SD* but lacking both inversions demonstrates that the latter are not essential for segregation-distortion either. These recombinants, however, are less stable (that is, have a greater range in *k* values and a somewhat lower mean *k* value) than the original *SD* lines. I shall return to this point later.

The *k* value of any particular heterozygous *SD* male, the distribution of *k* values expressed by the heterozygous *SD* brothers from any cross and the proportion of heterozygous *SD* males from any given cross that exhibits the *SD* effect may vary considerably according to the history of both the *SD* and the SD^+ chromosomes involved (Sandler and Hiraizumi, 1959).

PROCEDURE

Samples from wild populations of *Drosophila melanogaster* from various regions of the United States, including Hawaii, were analyzed. Males from these populations were crossed to *cn bw*; *e*; *ey* females, and from each pair mating two F_1 males (now heterozygous for a recessive marker on every autosome) were selected and individually testcrossed to *cn bw*; *e*; *ey* females. The F_2 progeny from each pair were then counted to detect any deviation from the 1:1 segregation ratio expected for each chromosome.

Seventy F_1 males from a population collected in the Cape region of Baja California in September, 1959, showed the following mean *k* values: chromosome I, 0.450; chromosome II, 0.685; chromosome III, 0.657; chromosome IV, 0.520. Approximately 16 per cent of these males exhibited second chromosome *k* values of 0.90 or above, while the other three chromosomes segregate normally. The slight preponderance of e^+ over *e* reflects nothing more

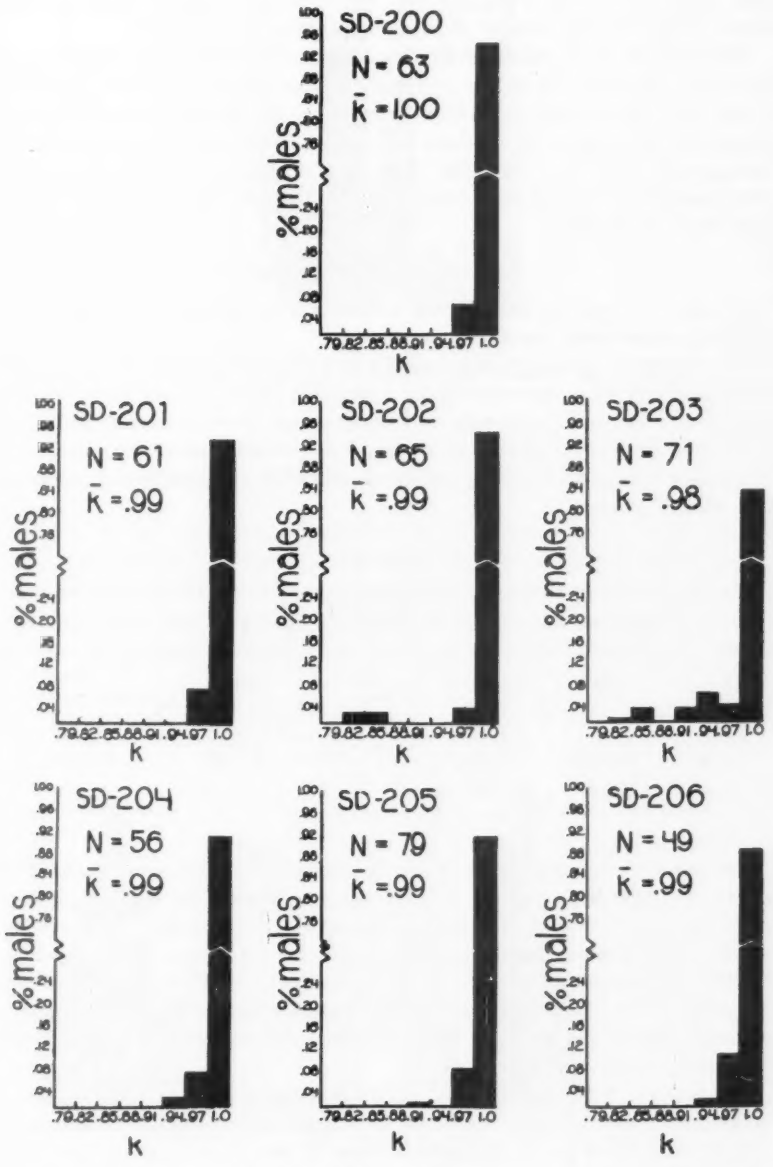

FIGURE 1. Summary of *k* values for seven *SD* chromosomes recovered from Baja California. *SD/cn bw*; *e/+* males are repeatedly backcrossed to *cn bw*; *e*; *ey* females.

than inviability of the *e* chromosome, which occurs in all tests; in no case was a relatively high *k* value of an e^+ male heritable.

The sons of all F_1 males exhibiting a second chromosome *k* value > 0.70 were testcrossed to *cn bw*; *e*; *ey* females; those lines with *k* > 0.90 proved to be very stable, and several were retained for further study. Figure 1 shows the distribution of *k* values from these stable high *k* lines, which are designated as *SD* lines 200–206. Each line represents a single *SD* chromosome recovered from the Baja population, but they are thus far indistinguishable from one another.

NATURE OF BAJA DISTORTER

In order to establish the precise nature of this aberrant chromosome, the following experiments were performed.

Expression by male sex only. As figure 1 illustrates, *SD* has been stably maintained for many generations by repeatedly backcrossing heterozygous *SD* males to *cn bw*; *e*; *ey* females. The reciprocal cross, however, yields *SD* and *cn bw* progeny in approximately equal proportions, as shown in table 1. The slight excess of *SD* chromosomes recovered (*k* = 0.545) is accounted for by viability differences.

TABLE 1

Test Baja *SD* for presence of inversion and expression of *SD* in females: progeny obtained from matings of $\frac{SD^+\ cn\ bw}{SD\ +\ +}$ females × *cn bw* males

Line	*SD*	*cn bw*	*cn*	*bw*	Total	Per cent recombination
200	12,374	10,591	7	15	22,987	0.0957
205	12,826	10,418	4	21	23,269	0.1070
Total	25,200	21,009	11	36	46,256	0.1016

Inhibition of SD effect by the Curly inversion. From a mating of *SD/cn bw* males to *In*(2LR)*Cy* $cn^2/cn\ bw$ females, F_1 males of the constitution *In*(2LR)*Cy* cn^2/SD were selected for mating singly to *cn bw*; *e*; *ey* females, and F_2 progeny were counted in order to determine *k* values of the F_1 males. Figure 2 shows the distribution of these *k* values. As described above with the original *SD* lines, *k* values are all approximately 0.50, indicating that close pairing in the region of *SD* is essential for expression of segregation-distortion in this case also.

Sensitivity. Since homozygous *SD* yields progeny in 1 : 1 ratios, an important test for identity between the new distorter and the original Madison *SD* is insensitivity of one to the other. Baja *SD* was tested against two Madison *SD* recombinants, one (*R*(*SD*-5)-18) bearing an inversion and the other (*R*(*SD*)-4) inversion free. (For the standard *k* values of these two recombinants, see Sandler and Hiraizumi, 1960.) Males heterozygous for the Baja *SD* chromosome (bw^+) and either of the two recombinant chromosomes (*bw*),

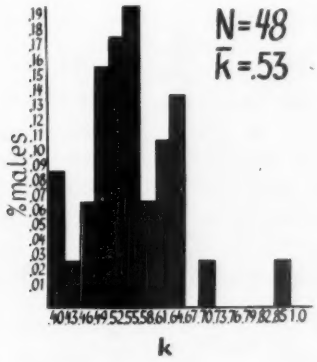

FIGURE 2. Distribution of k values of males of the constitution $\frac{SD}{In(2LR)Cy\ cn^2}$.

which were individually mated to *cn bw* females, yielded the distribution of k values represented in figure 3. Obviously, the segregation ratios are normal.

Presence of an inversion. *SD/cn bw* females from two Baja California *SD* lines were chosen and mated to *cn bw* males, and the F_1 parental and recombinant classes were tallied. Results are summarized in table 1. It is clear from the exceedingly low recombination frequency that at least one inversion is present on the Baja *SD* chromosomes, since *cn* and *bw* are 47 standard map units apart. The difference in recombination frequency between line 200 and line 205 is not statistically significant (chi-square value is 0.15, with probability of 0.5–0.7 for one d.f.).

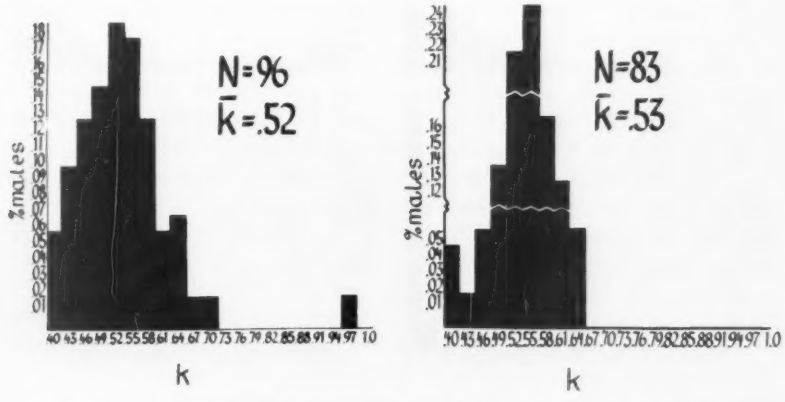

FIGURE 3. Test distortability of Madison *SD* [*R(SD-5)-18* with an inversion and *R(SD)-4* without an inversion] by the Baja *SD*. Distribution of k values from males of the constitution $\frac{SD\text{-}205\ bw^+}{R(SD\text{-}5)\text{-}18\ bw}$ or $\frac{SD\text{-}205\ bw^+}{R(SD)4\ bw}$.

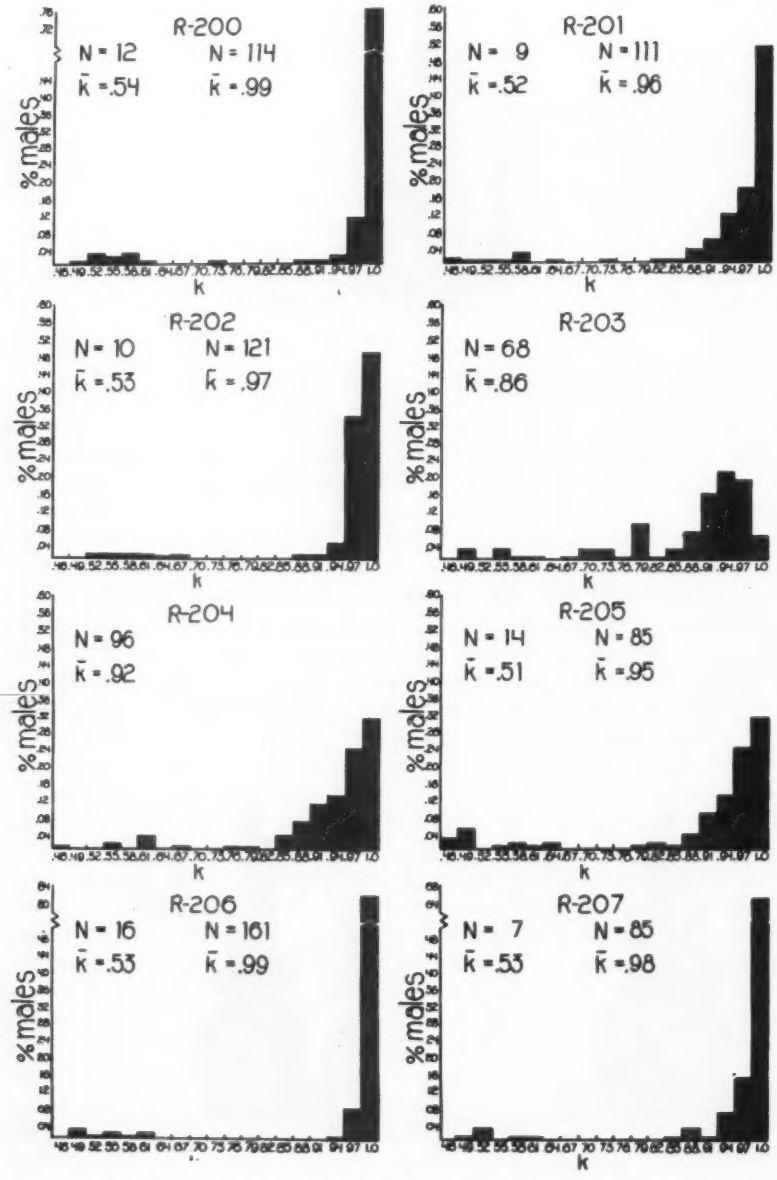

FIGURE 4. Summary of *k* values for eight recombinant *SD* chromosomes; lines R-200 through R-203 were derived from Baja *SD*-200, and lines R-204 through R-207 are derivatives of Baja *SD*-205. Males of the constitution *R-SD bw/cn bw; e/+* are repeatedly backcrossed to *cn bw; e; ey* females.

Male recombinants, both *cn* and *bw*, were mated singly to *cn bw*; *e*; *ey* females, and progeny were counted to determine *k* values. The *bw* recombinants carried the *SD* locus while the *cn* recombinants did not, indicating that *SD* must lie near the region of *cn*. Figure 4 presents a summary of *k* values for each *SD* recombinant line. It may be noted that most of the recombinants are less stable than the original *SD* chromosome, as has been seen in the case of the Madison *SD* recombinants (Sandler and Hiraizumi, 1960). A rather striking exception is recombinant 206, which is apparently stable—with occasional exceptional *k* values of approximately 0.50, the cause of which is not understood at present. Recombinant 200 is either semistable or stable.

TABLE 2

Test Baja *SD* recombinants for presence of inversion: progeny obtained from matings of $\frac{pr\ +\ cn\ +}{+\ SD\ +\ bw}$ females × *cn bw* males

Recombinant	*cn*	*bw*	++	*cn bw*	Per cent recombination
200	2654	2655	1552	1501	36.51
201	2492	2386	1281	1291	34.52
202	2595	2728	1538	1475	36.14
203	2522	2512	11	10	00.42
204	2334	2272	1357	1336	36.90
205	2200	2295	1246	1226	35.48
206	2310	2220	1383	1446	38.44
207	2391	2332	1188	1248	34.03

Mapping SD. The most crucial test of identity is the location of the putative Baja *SD* locus. The following experiment was designed to map *SD* and, in addition, to determine which recombinants carry the inversion. From a mating of *R-SD bw/cn bw* males × *pr cn* females, F_1 females of the constitution *pr* + *cn* +/+ *SD* + *bw* were selected and mated to *cn bw* males. Table 2 summarizes the results of the F_2 count for every recombinant line; low recombination frequencies indicate the presence of the inversion. Since seven of the eight *SD bw* recombinants were inversion free, the crossover most often occurs proximal to the inversion on the Baja *SD* chromosome.

In the F_2, $cn^+/cn\ bw$ male derivatives of recombinant line 206 were selected. The cn^+ chromosome would carry *SD* if no crossover had occurred between *cn* and *SD*, but would be SD^+ if a crossover had taken place in this region. These males were mated to *cn bw*; *e*; *ey* females. One $cn^+/cn\ bw$ son from each F_2 male was mated to a *pr cn* female; progeny were counted to determine *k* values, and also scored with reference to presence or absence of *pr*. In this manner, the frequency of crossing over between *cn* and *SD*, detected as the proportion of $cn^+\ SD^+$ chromosomes, was determined. The presence or absence of *pr* indicates whether *SD* lies to the left or to the right of *cn*. In a total of 539 chromosomes tested, no crossovers between *cn* and *SD* were found (that is, all were $pr^+\ SD\ cn^+$). Hence the Baja *SD*

locus is very closely linked to *cn*, as is true of the original *SD* (Sandler, Hirziaumi and Sandler, 1959), although lack of recombinants between *pr* and *cn* makes it impossible to state whether it would map to the left or to the right of *cn*. Nevertheless, it is most reasonable that Baja *SD* is allelic to the original Madison *SD*.

These data suggest, in addition, that crossing over is reduced in the Baja *SD* region, since *pr* and *cn* are 1.1 map units apart in these stocks. The nonrecovery of crossovers is significantly different (probability of less than 0.01) from this expectation.

DISCUSSION

It has been shown that the distorting chromosome discovered in a Baja California population of *Drosophila melanogaster* is indeed *SD*, as evidenced by the following properties: (1) expression of the aberrant segregation ratios in males heterozygous for this chromosome and a structurally normal SD^+ chromosome which is sensitive to Madison *SD*; (2) normal 1:1 segregation from a female of the same constitution; (3) insensitivity of Baja *SD* chromosomes to the distorting action of Madison *SD* chromosomes and vice versa; (4) location of the new distorter locus in or near the centromeric heterochromatin of chromosome II; (5) inhibition of segregation-distortion in *SD/Cy* males; and (6) lowered stability of recombinant *SD* chromosomes as compared to the original *SD* chromosomes from which they were derived.

The occurrence of *SD* in Baja California as well as in Madison suggests that the phenomenon of segregation-distortion is not of recent evolutionary origin. Furthermore, one would expect that if there has been geographical separation over a long period of time, differences between the two *SD*'s might have evolved. It has been noted that the behavior of Baja *SD* recombinants is in some respects dissimilar to that of the Madison *SD* recombinants; whereas all Madison *SD* recombinants are less stable than the *SD* chromosomes from which they were derived (Sandler and Hiraizumi, 1960), this characteristic pattern of instability is not always expressed in the Baja recombinants. It may be seen from figure 4 that two recombinants (R-203 and R-204) are unstable and four (R-201, R-202, R-205 and R-207) are semistable; recombinant 206 and possibly R-200, however, are apparently stable (mean $k = 0.99$) except for the occasional nondistorting males. Indeed, this peculiar bimodality clearly exists in the k value distributions of all of the recombinants (although not detectable with certainty in the unstable recombinants); thus, males that do not exhibit segregation-distortion (k = ca. 0.55) occur with a frequency of approximately ten per cent in all recombinant lines. This was observed in the mapping experiment also, where 55 of the 539 recombinant chromosomes tested gave k values of approximately 0.50 and had to be tested for another generation to determine that they were in fact *SD*. Such behavior is not manifested in any of the Madison *SD* stocks, or in the original Baja *SD* chromosomes. The genetic basis for these nondistorting males is at present not understood, but is being investigated.

It is possible that the higher level of stability exhibited by the Baja *SD* recombinants as compared with the Madison *SD* recombinants reflects a difference in the tester stocks used (*cn bw*; *e*; *ey* versus *cn bw*), rather than a difference in the *SD* loci themselves. Experiments to test this are now under way.

Detailed analyses by Sandler and Hiraizumi (in press) demonstrate that slight alterations in the substructure of the *SD* region may result in rather dramatic phenotypic changes. For example, they have been able to separate the *SD* region by crossing over into several component parts which, by appropriate recombination and manipulations can lead either to reconstitution of the *SD* phenotype again (giving an excess of *SD* chromosomes) or to the generation of a system wherein the *SD*-bearing chromosome acts upon itself rather than its homologue (giving a deficiency of *SD* chromosomes).

In this connection, a very small number of cases has been observed where a Baja *SD* chromosome was completely sensitive to the action of a Madison *SD* recombinant (see figure 3). Whether or not this sensitivity can be correlated with the occurrence of recombinants which do not exhibit segregation-distortion is not yet known; but it is possible that the explanation lies in small structural variations within the *SD* locus.

All *SD* chromosomes recovered thus far from nature have carried inversions. This is interesting, inasmuch as the frequency of inversions in wild populations of *Drosophila melanogaster* is very low (Ives, 1947). Since the fitness of populations infected by *SD* is considerably lowered due to the deleterious effects of the locus itself as well as the detrimental and lethal loci which may be linked to it, *SD* is maintained only by virtue of its high *k* values (Hiraizumi, Sandler and Crow, 1960). The evolutionary function of inversions, as crossover suppressors, in maintaining the stability of *SD*, is clear, although the mechanism is not understood. The presence of an inversion on the Baja *SD* chromosome suggests that a stabilizer may be located on the tip of this chromosome also; if so, it follows that the *SD* stabilizer locus is old too.

The apparent age of *SD* indicates that meiotic drive can indeed maintain alleles which are selectively disadvantageous in a population. The T-loci in mice, whether or not they represent an instance of meiotic drive, nonetheless are undisputably deleterious. Dunn (1953) has shown that these loci also exhibit abnormal segregation ratios and remain in high frequencies in many populations of house mice.

Further studies on the geographical distribution of *SD* are contemplated.

SUMMARY

The phenomenon of segregation-distortion, originally discovered in Madison, Wisconsin, has now been detected in a natural population of *Drosophila melanogaster* from Baja California. It has been demonstrated that the second chromosome responsible for the aberrant segregation is an *SD*-bearing chromosome, because it shares the following properties with the Madison *SD*:

(1) It is expressed in the male sex only. (2) It is inhibited by the *Curly* inversion. (3) The Baja California *SD* chromosome is not sensitive to the action of the Madison *SD* chromosome. (4) It is located near the centromere (closely linked to *cn*).

This finding suggests that *SD* is not of recent origin.

ACKNOWLEDGMENTS

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SPONTANEOUS CHROMOSOME ABERRATIONS IN HUMAN TISSUE CULTURE CELLS

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The use of mammalian tissue culture cells to measure the effects of x-ray and other mutagenic agents on the cells *in vivo* must be used with caution. Puck (1960) found that the mean lethal x-ray dose for human fibroblast cells grown in tissue culture was approximately 50 r, while the L. D. 50 for the human species is 400–600 r. An x-ray dose of 50 r produced chromosome aberrations in most of the cells and he concluded that chromosome damage could account for most, if not all, of the death or loss of reproductive ability in irradiated human tissue culture cells. Puck also found a high incidence of spontaneous chromosome aberrations in the cells grown *in vitro*. On the other hand Bender (1959) found that 50 r of x-rays produced chromatid aberrations in only 4.7–37.5 per cent of his cultured "epithelioid" cells, depending on the duration of time between irradiation and fixation of the cells. Bender found very few spontaneous chromatid aberrations, ranging from 0 to 1.2 breaks per 100 cells, in these cultures which were grown in modified Chang's medium.

It seems improbable that such differences in the frequency of spontaneous chromosome aberrations, and in x-ray sensitivity, could be due to the tissue used for the cultures. Differences in spontaneous aberration frequency and sensitivity to x-rays can also be attributed to differences in the method of scoring the types of aberrations, the treatment of the tissue in culturing, the composition of the culture medium and the age of the culture.

Puck (1958) found spontaneous chromosome aberrations in about 20 per cent of the cells in his fibroblast cell cultures, most of them single chromatid deletions. But many of the aberrations scored at metaphase were probably achromatic lesions which would not lead to chromatid deletions. Chu and Giles (unpublished) found chromatid aberrations in about ten per cent of the fibroblast cells grown in Puck's medium. These differences could be due to differences in the method of scoring.

The effect of the culture medium on spontaneous chromosome aberrations was shown by Levan and Biesele (1958). They found spontaneous chromosome aberrations in about ten per cent of their mouse tissue culture cells. These aberrations included single and paired chromatid deletions and chromatid bridges, with or without fragments. There was some evidence of increased aberration frequency with the age of the culture, but there was great variability in the aberration frequency in different cultures of the same age. These cultures were treated with trypsin. In cultures which had not been

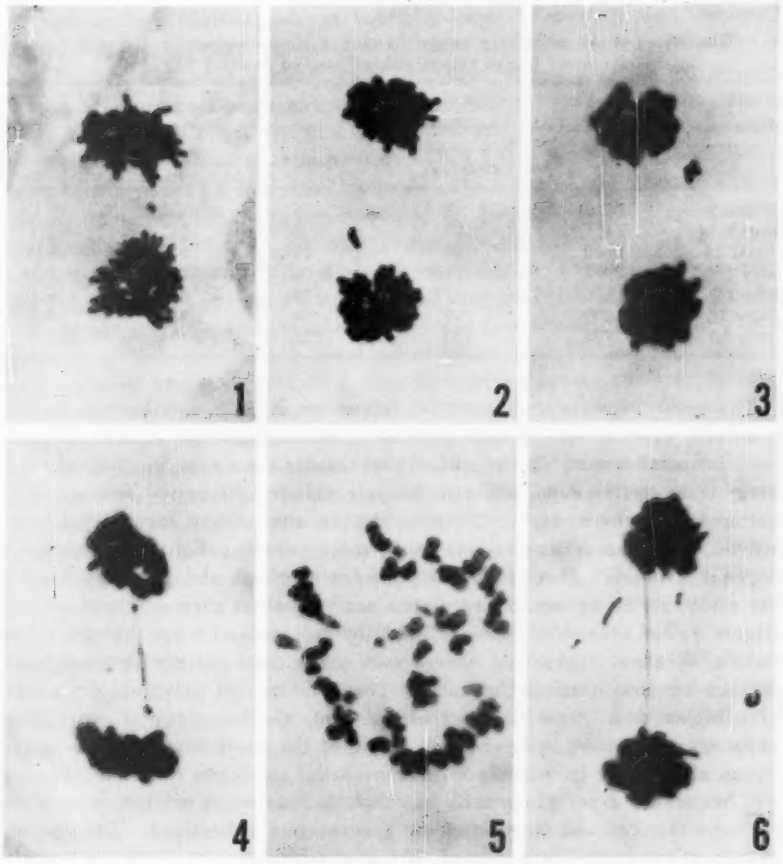
treated with trypsin the frequency of abnormal anaphases was greatly reduced—4.7 per cent compared with 12.6 per cent in the trypsin treated cultures.

The effect of the culture environment was also shown in rat sarcoma cells. Hsu and Manna (1959) found that 40 per cent of the cells showed chromatid aberrations when the tissue was grown in a culture medium with no asparagine, while few were found in cells grown in a medium containing asparagine.

In testing suspected mutagenic and carcinogenic agents on mitotic aberrations in human tissue cultures, it was found that the frequency of spontaneous aberrations increased with the age of the culture. The sensitivity of these cultured cells to x-rays also seems to be related to the age of the culture.

Our cultures were from prepuces from newborn infants obtained from the Yale Medical School. The tissue was mascerated, treated with 0.2 per cent trypsin at room temperature in a magnetic stirrer for an hour. The cultures were grown in Puck's (Puck, Cieciura and Robinson, 1958) fibroblast medium and subcultured every three to seven days. For cytological studies the cells were seeded into Leighton tubes with 30 × 11 mm. cover slips and incubated at 35° or 37°C. The cells on the coverslip were fixed in 3:1 alcohol acetic acid, rinsed in distilled water, air dried 24-48 hours, stained in two per cent orcein in 45 per cent acetic acid, and dehydrated in tertiary butyl alcohol, transferred through alcohol-xylol and mounted in Canada balsam.

Chromosome aberrations were scored at anaphase and telophase. Analyses at these stages are not as precise as scoring metaphase chromosomes following colchicine treatment, but the scoring can be done much more rapidly and for comparative purposes is quite adequate. The types of aberrations found were dot (figure 1) and rod deletions, either single (figure 2) or in pairs (figure 3), and chromatid bridges, with or without (figure 4) acentric fragments. The acentric rod and dot deletions are presumably chromatid deletions, involving one or both sister chromatids, while the chromatid bridges appear to be the result of isochromatid deletions followed by fusion of the broken ends of the centric chromatids. The chromatid bridges should be associated with acentric fragments, but the frequent absence of fragments associated with bridges could be due to the inclusion of the fragment among the normal chromosomes at the poles. At any rate the bridges show no evidence of resulting from half-chromatid exchanges since there is no evidence of distal chromatid arms projecting from the center of the bridge. There were occasional double bridges which might have resulted from chromosome dicentrics, but it is more probable that they were two independent chromatid bridges closely associated. The absence of any obvious chromosome type aberrations is in accord with more precise analysis of spontaneous chromosome aberration at metaphase made by Chu and Giles. They found that all, or nearly all, of the spontaneous aberrations occur at prophase, when the chromosomes are differentiated into chromatids.



Photographs of chromosome aberrations in human tissue culture cells.
Magnification, × 1500

FIGURE 1. Spontaneous dot deletion.
FIGURE 2. Spontaneous single chromatid rod deletion.
FIGURE 3. Spontaneous double rod deletion.
FIGURE 4. Spontaneous chromatid bridge.
FIGURE 5. Metaphase with contracted chromosome due to culture medium.
FIGURE 6. X-ray induced chromatid bridge plus rod and dot deletions. 25 r.

During the period of analysis it became evident that the frequency of spontaneous aberrations increased with the age of the culture. The culture used in these experiments was started on January 13, 1960, but experimental tests were not started until March 8, and were continued into July. As is shown in table 1, the frequency of cells with chromosome aberrations increased consistently from 3.6 per cent in March to 9.6 per cent in June.

TABLE 1

The effect of age of culture on the frequency of spontaneous chromosome aberrations in human tissue culture started January 13, 1960

Date of fixation	Total cells	Cells with anaphase aberrations			
		Rod and dot deletion	Bridges	Total	Per cent
March 3	2818	98	4	102	3.6
March 14	2471	117	8	125	5.1
April 13	1740	100	18	118	6.8
May 5	1927	113	24	137	7.1
May 19	1390	97	16	113	8.1
June 15	2899	219	58	277	9.6
July 10	772	127	28	155	20.1

The great increase in aberration frequency in July may be due to the relatively small sample of cells, but more probably it is due to more extreme environmental factors. In the earlier experiments the tissue was subcultured every three to five days, while in the July cultures the period between subcultures was seven days. Other factors on environment may also be involved, but whatever the factors were, it was evident that the environment was not favorable. There were relatively few anaphase and telophase figures per slide; all of the metaphase plates had very short compact chromosomes (figure 5) and resembled those affected by colchicine except that the chromatids were not separated. There were many dead cells with amorphous masses of dark staining chromatin. The incidence of polyploid cells was also higher than in earlier cultures, in which the frequency of polyploidy cells was less than one per cent. Some of the sharp increase of spontaneous aberrations in the July culture might be attributed to polyploidy, but the frequency of polyploid cells at anaphase was much too low to account for more than a small fraction of the spontaneous aberrations. The consistent increase in the frequency of aberrations with the age of the culture indicates that culture age is a major factor in the incidence of spontaneous chromatid aberrations.

There is also evidence that the older cultures are more sensitive to x-rays. In a series of four experiments the control slides had a high incidence of spontaneous chromatid aberrations—198 in 2104 cells, or 9.4 per cent. In the 1000 cells which had received 25 r of x-rays 23-25 hours before fixations, 548, or 54.8 per cent, had aberrations at anaphase. Thus the frequency of cells with aberrations caused by the x-rays was 45 per cent. Even if all of these aberrations are "one-hit" types, a dose of 50 r would be expected to produce aberrations in 90 per cent of the cells, practically all of the cells, if a significant number of the aberrations are of the "two-hit" type. These results are in accord with Puck's observations. The spontaneous aberrations were usually limited to one per cell, but an x-ray dose of 25 r often produced several aberrations in a single cell (figure 6).

Although we did not subject young cultures to x-irradiation, Bender's (1959) results indicate that younger cultures are not only less sensitive to x-rays, but also have a much lower incidence of spontaneous aberrations. His epithelioid cells from human kidney tissue were irradiated after two to five transfers following the original culture and fixed at 42–49 hours after irradiation. The control cells showed only 1.0–1.2 per cent of spontaneous aberrations and 25 r of x-rays produced only 6.1–17.5 per cent of aberrations. In monkey kidney tissue culture cells, he found only 1.5 spontaneous aberrations per 100 cells and only 12.0 following a dose of 25 r of x-rays. In monkey bone marrow cells *in vivo* no spontaneous aberrations were found in 261 cells, and 25 r of x-rays produced only two aberrations in 50 cells, or four per 100. No spontaneous aberrations were found in 181 bone-marrow mitoses in hamsters, but in established cultures of "fibroblast" cells of the Chinese hampster, obtained from George Yerganian in Boston, there were spontaneous aberrations in about 20 per cent of the cells and 46.2 aberrations per 100 cells following an x-ray dose of 25 r.

It is evident that environmental factors can greatly increase the incidence of spontaneous chromosome aberrations of mammalian cells grown in tissue culture, and also increase the sensitivity of these cells to x-rays. It is possible that different tissues vary in the incidence of spontaneous chromosome aberrations due to internal environmental factors. Giles (1941) found that in Tradescantia microspores spontaneous chromosome aberrations were about 12 times as frequent in triploids as in diploids. The difference was attributed to the unbalanced genomes in the triploid since no differences were found in root tip cells. Variation in spontaneous chromosome aberrations were also found in different diploid Trillium plants. Sparrow and Sparrow (1950) found that the frequency of micronuclei at microspore interphase ranged from 0.25 to 3.56 per cent in different plants.

It is possible that "epithelioid" and "fibroblast" cells cultured from mammalian tissue differ in frequency of spontaneous aberrations and sensitivity to x-rays, but it seems probable that great differences can also be attributed to sensitivity of the cultured cells to the tissue culture media, relative growth rates and age of cultures. Even the less sensitive "epitheloid" cells usually have a much higher incidence of spontaneous chromosome aberrations than can be expected *in vivo* cells. The conclusion of Levan and Biesele (1958) that "even with the mildest condition of preparation, newly set up tissue cultures will exhibit a considerable frequency of mitotic disturbances," must be considered in any comparison of spontaneous and x-ray induced chromosome aberrations *in vivo* and *in vitro*.

SUMMARY

Spontaneous chromosomal aberrations may occur with a high frequency in human tissue culture cells, and the frequency increases with the age of the culture. In a series of experiments the frequency of aberrations found at anaphase and telophase increased from 3.6 per cent to 9.6 per cent as the

culture aged over a period of three months. Most, or all, of the aberrations appear to be of the chromatid type, and must have originated at prophase of the nuclear cycle. There seems to be some correlation between the frequency of spontaneous chromosome aberrations and sensitivity to ionizing radiation in mammalian tissue culture cells.

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THE DIFFERENTIAL SUSCEPTIBILITY OF SPERM AND SPERMATIDS TO IONIZING RADIATIONS

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If different stages of developing germ cells are exposed to ionizing radiations, the number of mutations which are obtained from these gametes in a sequence of mating periods varies to a large extent. In the male germ line, the fewest mutations are always obtained from irradiated spermatogonia, much more from spermatocytes and spermatids and an intermediate number from fully developed sperm. This phenomenon is widely spread in the animal kingdom; it is not only well known in Drosophila but also in vertebrates as for example in mice (Russell, 1952; Bateman, 1958) and certainly it also involves man.

There are an unexpectedly large number of problems hidden in this simple fact. As long as the immature gametes are subject to mitotic and meiotic cellular divisions, the reasons for the differential yield of mutations from these cells are very complex. We should mention a few of them in order to prepare the ground for the following discussion concerning postmeiotic stages. Regarding the rate of dominant lethal mutations, there is no doubt that a considerable part of the radiation induced chromosome breaks, after going through cellular divisions, result in severe aneuploid conditions which can prevent the cells from becoming functional sperm; this, of course, gives rise to fewer detectable dominant lethals. It is another question whether there is a so-called germinal selection for the kind of genetic damage which usually is no obstacle to chromosomal divisions. The most interesting and, practically, the most important problem involves the very low mutation rate obtained from spermatogonia, especially as it concerns presumed point mutations: recessive lethals (references reviewed by Muller, 1958) and recessive, visible, cytologically analyzed point mutations (Alexander, 1960). Is this due to an actual insensitivity of the chromosomes in this stage, or to germinal selection, or is the problem perhaps biased by an abnormally high sensitivity of the chromosomes of meiotic and postmeiotic stages? Compared to this problem, the differential radiosensitivity of sperm *versus* spermatids is of a rather limited importance, though it is quite interesting because it involves many basic features of radiation genetics.

During the development from spermatids to sperm no cellular divisions take place, but a very marked differentiation occurs in size, shape, cellular organization and in the chemical constitution. The spermatid at the beginning has a globular nucleus which develops in the course of its differentiation into the small rod-like or leaf-like head of the adult sperm. This head, which contains the chromosomes, has a much smaller volume than the nuclei

of preceding spermatid stages. (For Drosophila the pictures in Demerec, 1950, page 11, give an impressive illustration of the morphological changes.) The chromosomes are in a condensed state in both kinds of cells, more so in sperm than in spermatids.

This state of condensation brings about the first problem: it has been demonstrated in numerous plants and animals that the condensed chromosomes as they are found during mitosis and meiosis are the most sensitive to damage by radiation. However, it is questionable if the condensed chromosomes during cellular divisions can be compared to the condensed chromosomes during spermiogenesis. During the cellular divisions several simultaneous factors occur which are not present in spermiogenesis, particularly the extreme movements of the chromosomes taking place from prophase to anaphase, as well as the splitting of the chromosomes and their attachment to the spindle fibers. If the assumption is right that the condensation alone makes the chromosomes more susceptible to damage by radiation, this would be a factor superimposed on the problem of the differential sensitivity of spermatids *versus* sperm, because both have this condition. On the other hand, it would help to explain the lesser sensitivity of the spermatogonial stages as compared to postmeiotic stages with regard to the production of point mutations. But, first, the proof should be brought forward that condensation alone really is a determining factor.

Under usual conditions an irradiation with x-rays in air gives up to several times more genetic damage in spermatids than in sperm. In one of the two cell types there must be a special condition, capable of modifying, increasing or decreasing the effect of a given x-ray dose. In order to resolve the preliminary question as to which one of the two kinds of cells has this special property, we may look at their responses when we modify some experimental conditions. Then we will find between sperm and spermatids:

1. A difference in response to changes of the x-ray doses.
2. A different response to irradiation under anoxic conditions and under the influence of enzyme poisons.
3. A much smaller difference in the response to neutrons than to x-rays.

The different dose response has been observed for the production of dominant lethals and translocations in *Drosophila virilis* (Stone, 1956). By doubling the x-ray dose, damage in sperm always increases less than it does in spermatids (or in spermatocytes) (figure 1).

The difference in response of the two kinds of cells to anoxia is well known and many authors have pointed out this fact. In contrast to the overwhelming majority of investigated cells of animals and plants, which after irradiation in air show a damage two to three times greater than after irradiation in anoxia (reviewed by Gray, 1959), sperm, in contrast to spermatids, react extremely little to this change. This has been shown in Drosophila for the production of dominant and recessive lethals and for translocations. In this respect there is no doubt that sperm are the exceptional cells and

not the spermatids. The same is the case for the results obtained with enzyme poisoning with carbon monoxide (Schmid, 1961).

The third point, the differential response to neutrons, has been shown by Alexander (1958 a, b) in *Drosophila virilis*. The difference in sensitivity to neutrons between sperm and spermatids is much smaller than the one observed with x-rays. A given neutron dose which produces the same amount of damage in spermatids as a dose of x-rays, causes a higher damage in sperm than do the x-rays. This is true in air, oxygen, and, most strikingly, under anoxic conditions. Especially interesting is a result obtained by Alexander (1958 b) with 14 Mev-neutrons in nitrogen where, for the production of dominant lethals, every difference between sperm and spermatids has disappeared, although there is still a small difference in the production of translocations.

What are the explanations which are usually offered to account for the different response of sperm and spermatids? One favorite idea says that there are differences in the oxygen content in the two kinds of cells, due to differences in the activity of the cellular metabolism. That this cannot be the whole explanation has already been stated by Oster (1959) who concluded that oxygen "... is probably not the only factor responsible for the differential radiosensitivity ... since it was found that spermatozoa treated in pure oxygen are not as sensitive to the same dose of x-rays as spermatids treated in air." One might add here that it is still impossible to eliminate the difference by offering pure oxygen under a pressure of ten atmospheres (Chang, Wilson, and Stone, 1959). Further, an irradiation with x-rays in complete anoxia always gives more damage in spermatids than in sperm.

Other explanations concentrate on the high sensitivity of spermatids which probably is the wrong approach, since, as we have pointed out before, it is the sperm and not the spermatid which differs in its radiation response so much from other kinds of investigated cells (meristem cells, tissue culture cells, etc.). Such explanations assume that the genetic material in spermatids should be especially vulnerable because of DNA-replication or because of a change of nucleoprotein-histones to protamines. We will return to this change of the chromosomal proteins at the end of this paper.

In view of the above, one is well justified in seeking the special property or properties which are able to modify the effect of a given x-ray dose in *sperm*.

It has been shown (for example, Bloom, Zirkle, and Uretz, 1955) and is generally accepted that shortlived radiochemical products arising in the cytoplasm of a cell with a resting nucleus usually do not hurt the chromosomes, because the distance to the chromosomes is too great and such products are made innocuous by other constituents of the cell. But it is believed that these radiochemical products arising within a rather short distance from the chromosomes can reach the sensitive structures and cause damage in addition to that due to ionizations directly within the boundaries of the chromosomes.

This suggests the idea that perhaps, in the small head of the sperm, a considerable reduction of the space surrounding each chromosome might take place and be responsible for the observed different responses of sperm and spermatids. In order to investigate this hypothesis we have tried to explain the observed facts on this new basis and we have also examined electronmicrographs since they might indicate something in favor of our assumption. We have found support from both directions. Concerning the electronmicrographs we did not have available pictures from Drosophila, but since the phenomenon is very general among animals, it is as well justified to use pictures of vertebrates, especially since the changes from spermatids to sperm in the latter are not more but less extreme than in Drosophila. The changes which are observed on electronmicrographs of spermiogenesis of the toad, for instance (Burgos and Fawcett, 1956), are mainly the following: on cross-sections of early spermatids the chromosomal material is visible within the globular nucleus in the form of many tiny grains. In the still large nuclei of more advanced stages of spermatids, this chromosomal material becomes visible as fewer but bigger and denser particles, an expression of the chromosomal condensation taking place. The condensation is intensified for a while, but then, in progressively smaller nuclei, the space between the condensed chromosomes actually begins to diminish and in the differentiated sperm heads, the condensed chromosomes are lying tightly together in the form of a conus, with almost no space left between them.

But the answer to our question as to whether the proposed hypothesis fits the observed radiogenetical facts is more important than these observations.

The first observation we mentioned above, concerning the differential dose response, can be explained very well with this theory. The observed steeper effect dose curve of the spermatids as compared to sperm (figure 1) can theoretically be explained with an enlargement of a given target volume by a factor of three (figure 2). Now, of course, it is clear that the ionizations arising in a space around each chromosome are less effective in causing mutations than those arising within the chromosomes. The farther away the ionizations occur, the smaller is the chance that the produced active radiochemical compounds will reach the chromosome. Certainly this space demands more than three times the volume of the chromosome; its limits will be given by the greatest migration distances of the primary and secondary radiochemical products. It is generally accepted that these distances are greater than the extremely small distances observable on the electronmicrographs between the condensed chromosomes of the sperm. For primary radicals the migration distance is indicated by radiochemists as 10 to 100 Angstrom (Gray, 1958), but for secondary products, especially those induced in the presence of oxygen, this distance is believed to be considerably longer.

The second observation concerns the differential response to oxygen. Here we first must consider more detailed results obtained with spermatids and sperm. Oster (1957, 1959) has investigated this problem very carefully

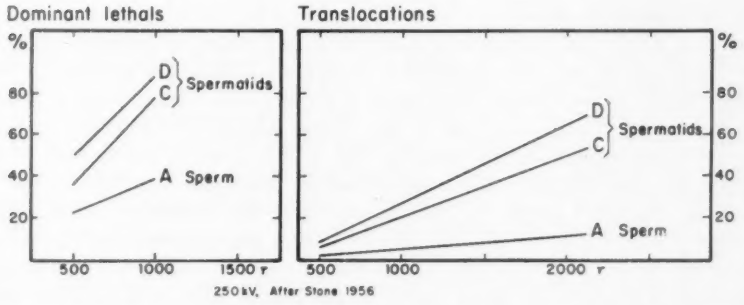

FIGURE 1. Differences in x-ray dose response of sperm and spermatids concerning the production of dominant lethal mutations and translocations. Sperm reacts much less to changes in the x-ray doses than spermatids. After Stone, 1956.

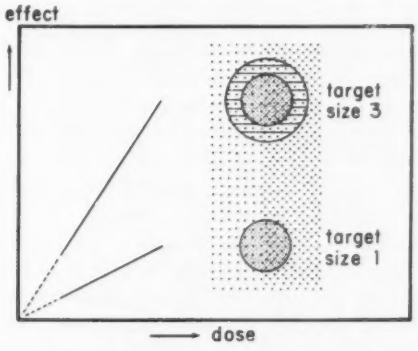

FIGURE 2. Dose response with different sizes of targets. A target three times greater than another target profits three times more from an increase in the dose of equally distributed events (for example, dose of ionizations).

in nitrogen, air and oxygen using as a measure the production of translocations (Oster, 1957) and recessive lethals (Oster, 1959) in *Drosophila melanogaster.* The results he found in nitrogen and air show a much greater difference in the amount of damage in spermatids than in sperm. But if the values obtained in air are compared to those in oxygen, about the same absolute increase of damage is observed in sperm as in spermatids. In other words, the first 21 per cent of oxygen is responsible for the large "oxygen effect" in spermatids, just as has been observed in so many other kinds of cells and to which an addition of oxygen up to 100 per cent adds relatively little more effect. But in sperm, the much smaller oxygen effect is more nearly proportional to its share in the environmental gas, from zero to 100 per cent. To explain these facts with our proposed theory, we have to assume that oxygen has more opportunity to interfere with the primary products

of the radiations outside of the chromosomes than inside. One may visualize that within the chromosome there are more compounds ready to undergo an oxidation or reduction (mutagenic or not) than there are outside in the caryolymph. A reasonable explanation of Oster's observations can be given, if we assume that interactions of the primary products with oxygen outside of the chromosomes are so frequent that a saturation is reached at 21 or fewer per cent oxygen. More oxygen has no enhancing effect here, but it still has an effect within the chromosome where the oxygen-dependent processes are less frequent and the system cannot become saturated up to 100 per cent environmental oxygen. This would give the reason why sperm and spermatids react to the same extent to the change from air to 100 per cent oxygen. The space surrounding the chromosomes of the spermatids would be very important for the first 21 per cent of oxygen, additional oxygen would become ineffective within this space because of an exhaustion of the processes susceptible to an interaction with oxygen.

Finally we come to the third point, the much smaller difference in response of sperm and spermatids to neutrons. From dozens of experiments the higher RBE-values (Relative Biological Efficiency) of densely ionizing radiations are well-known to every radiation biologist. All these observations reporting the very high efficiency of alpha-particles, protons or neutrons, depend directly or indirectly on the effect of these radiations on the chromosomes of the treated material. But no higher efficiency of densely ionizing radiations (measured as absorbed energy) is observed when the effect on chromosome-independent reactions is measured, for instance the inactivation of enzymes by irradiation; here, in contrast, x-rays are even much more effective than the densely ionizing radiations (Dale, Gray, and Meredith, 1949). It is most probably the very dense local concentration of ionizations in the tracks of neutrons which is so dangerous in connection with chromosomes. In regard to our theory we are tempted to say that a dense ionization track of a neutron either passes through a chromosome, in which case it will most probably cause damage, or it passes by the outside of the chromosomes where its absorbed energy is not more dangerous than the corresponding energy absorption from x-rays. As we stated above, in order to get an equal amount of chromosome damage, we need more absorbed energy from x-rays than from neutrons. Of both doses a small and probably equal percentage will be absorbed within the chromosomes and the rest will be absorbed outside the chromosomes. This remainder, measured as absorbed energy, will, consequently, be much greater in the case of x-rays than in the case of neutrons. Space outside the limits of the chromosomes, therefore, cannot play an important role for neutrons. Similar considerations apply to the small influence of oxygen on the action of the neutron-irradiation.

We did not take into account in our hypothesis possible chemical changes in the composition of the chromosomes or their environment during the differentiation of the spermatid. In the Drosophila literature it has been proposed (for example, Stone, 1956; Oster, 1959) that a replacement of histones

by protamines in the chromosomes during spermiogenesis might be responsible for the differential susceptibilities to x-rays. It has been proposed that the protamines due to their greater number of basic NH_2 groups could afford protection for spermatozoa during irradiation. We have some objections to this assumption. First, this protein transformation has been shown only for a very limited number of animals, for many kinds of fishes and a few years ago also for the rooster (gallus). It is only suspected to occur partially in mammals (Felix, Fischer, and Krekels, 1956) and in insects the situation is fully unknown. Second, if we assume that this protein change actually is a general process in every spermiogenesis, it would be as easy to visualize that at the end of this change there would be not more but less free basic NH_2-groups, for protamines have much smaller molecules than the histones. The molecular weight of protamines is indicated as 2,000 to 12,000 (Felix, Fischer, and Krekels, 1956) and of the nucleohistones as ca. 2,000,000 (Carter, 1951). Further it would be very hard to explain all the observed differences in radiation response on the mere basis of such a change in the nucleoproteins; especially, the complicated behavior in connection with oxygen and the response to neutrons would cause great difficulties.

To answer another objection, we admit that some restitution processes might interfere in the conditions leading to the differential radiation sensitivity of sperm *versus* spermatids. Thus, such a restitution process could not be identical with the known ATP-energy-dependent chromosomal reunion process, first, since restitution, in our problem, against all evidence, would have to be more effective in sperm than in spermatids, and second, because the differential susceptibility to radiations involves one-hit mutations (most of the recessive and dominant lethals) as well as multi-hit mutations (for example, translocations). Therefore, one would have to assume a still unknown kind of ATP-independent reunion processes, favoring the original rearrangement of broken chromosomes. It is not improbable that such a type of reunion process may exist, but it would be also very difficult to explain on this basis all the observed peculiarities in the radiation response of sperm and spermatids.

SUMMARY

A hypothesis is offered to explain the differential susceptibility of sperm and spermatids to ionizing radiations. In Drosophila, as in higher animals, a given x-ray dose causes up to several times more genetic mutations in spermatids than in sperm. Further, the two kinds of cells show different dose effect curves, react differently to changes in the oxygen tension during x-irradiation, but behave very similarily to neutron irradiation.

In sperm the condensed chromosomes are packed tightly together in the small sperm-heads, while the chromosomes of spermatids, located in regular nuclei, are separated from each other by some space within the caryolymph. The proposed hypothesis assumes that the diffusing secondary radiochemical products of ionizations, occurring in the immediate neighborhood of the

chromosomes, cause a considerable amount of chromosomal damage in addition to the effects due to ionizations occurring within the structures of the chromosomes themselves. The additional space is present around the chromosomes of spermatids as in most other types of cells, but it is practically missing in the exceptional cell-type represented by sperm. On the proposed basis it seems to be possible to explain not only the lesser sensitivity of sperm to x-rays but also the peculiar reaction to oxygen and to neutrons. Some other propositions to account for the differential susceptibility are discussed briefly.

ACKNOWLEDGMENTS

I wish to thank Professor Wilson S. Stone and Dr. A. C. Fabergé for reading this manuscript and for their criticisms. I also wish to express my appreciation to Dr. M. R. Wheeler for his linguistic help.

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NATURAL SELECTION ON A SPONTANEOUS INVERSION*

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The balanced polymorphism of inversions on the third chromosome of *Drosophila pseudoobscura* has become a classic case of the maintenance of genetic heterogeneity in local populations, and has been studied from many points of view (Dobzhansky, 1951; numerous later papers are mainly in *Evolution*). From the standpoint of population genetics, these inversions may be treated as alleles. They can readily be identified in salivary gland preparations. In the course of an extensive joint investigation with L. Levine, O. Pavlovsky, Th. Dobzhansky, and J. Beardmore on the effects of temperature on a pair of these inversions, to be reported elsewhere, a new inversion appeared spontaneously in one of my cages. In conformity to the practice of naming inversions in this species after their place of discovery, this inversion may be called Morningside (MO), after Morningside Heights in Manhattan.

MO differs by a simple inversion from Chiricahua (CH), and was obviously derived from the latter. No other reported inversion would make a simple heterozygote with it. The distal break is in section 80B, the proximal one in 71A (figure 1), using the terminology of Dobzhansky and Sturtevant (1938). The proximal break is unique, but the distal one seems similar to those in Olympic and Pinon (Epling and Lower, 1957).

The only previous inversion of *D. pseudoobscura* known to have occurred in the laboratory is one in the fourth chromosome found in a single individual by Dobzhansky (1956). The radiation-induced inversion tested by Sperlich (1959) in *D. subobscura* was found heterotic with all three other inversions tested. Thus no previous case has shown a pattern of selection similar to the present one.

The population where it appeared was maintained in a lucite population cage at 22° ±1°C; egg samples were taken at intervals and the larvae raised under optimal conditions. For further experimental details see Levine (1955) and the forthcoming paper on temperature effects.

The cage was started in January, 1958, with a frequency of 80 per cent CH and 20 per cent Arrowhead (AR) chromosomes. In the first sample, three generations later, two individuals out of nine on the same slide were heterozygotes between MO and CH. Four hundred and four third chromosomes were examined instead of the usual 300, but no further examples were found. Therefore they could well have been derived from eggs laid by the same mother.

*Data obtained during the tenure of two National Science Foundation predoctoral fellowships, 1957–1959, and a Columbia University fellowship, 1959–1960.

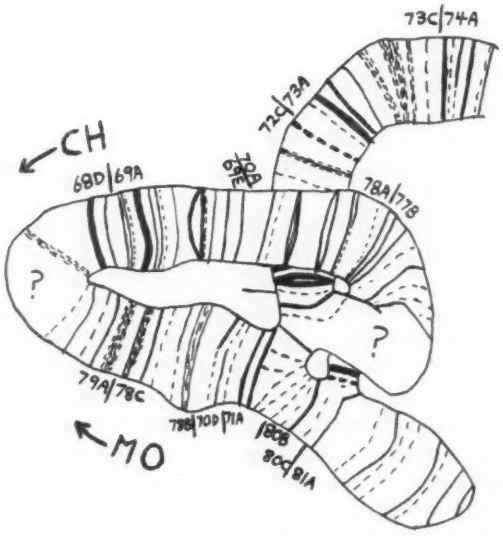

FIGURE 1. Distal end of the third chromosome in a heterozygote between MO and CH. The arrows point toward the proximal end of the respective chromosomes. Question marks denote unstained areas. Only a single cell was used, as staining and therefore apparent band patterns vary markedly from cell to cell.

The results of subsequent samples can be seen from table 1 and figure 2. The frequency of MO increased to about five per cent at eight generations, and then began a slow but steady decline until its final virtual or complete elimination at 33 generations. The frequency of CH showed a generally parallel pattern. It declined rapidly at first to 58 per cent at eight generations, but then reversed itself and reached a maximum of 70 per cent at 13 generations, after which it again declined to a final frequency of 39 per cent at 33 generations.

These changes in frequency can be at least qualitatively explained by a simple model. At 22°, AR homozygotes are known to have a much greater relative fitness than CH homozygotes, and therefore to increase in frequency at the expense of the latter (unpublished data). This accounts for the early rapid decline in the frequency of CH. If we now assume that the MO/CH heterozygote was heterotic in this environment, this would increase the relative fitness of CH, particularly if the MO/AR heterozygote were less fit than the CH/AR karyotype. This might explain the extremely rapid rise of MO (by a factor of about ten in five generations at low frequency), as well as the reversal in the direction of selection between CH and AR, which occurred simultaneously with the maximum frequency of MO.

A delayed decline in the relative fitness of both MO and CH could be brought about to my knowledge by only one mechanism, which has been proposed on other grounds (Wallace, 1953), in addition to hypothetical *ad hoc* genic or environmental changes. This envisions the occasional occurrence

TABLE 1

Changes in the observed frequencies of the third chromosome types. The modal generation length is 29 days.

Generations after start	Per cent AR	Per cent CH	Per cent MO
0	20.0	80.0	0.0
3.4	27.7	71.8	0.5
8.2	37.3	58.0	4.7
11.0	35.0	63.0	2.0
13.4	28.7	69.7	1.7
16.8	32.0	67.3	0.7
22.2	43.3	56.3	0.3
29.6	53.0	46.7	0.3
33.3	60.7	39.3	0.0

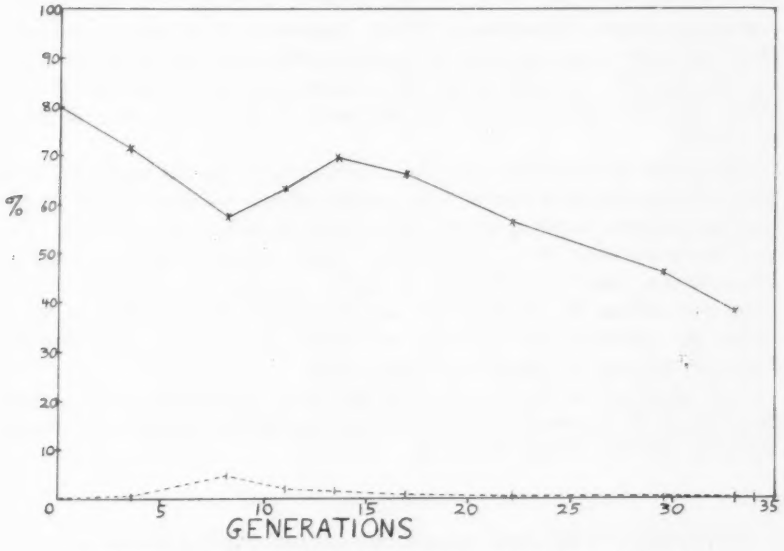

FIGURE 2. Graph of the observed frequencies of CH (solid line) and MO (dashed line).

of double crossing over within the long and simple MO/CH inversion loop, which would lead to viable eggs. This process, however, would transfer some CH genes to MO and vice versa. This in turn should lead to a reduction in the heterosis between them, since many structural heterozygotes would eventually be in part of their length genically as homozygous as either structural homozygote. There is, unfortunately, no basis for making a quantitative estimate of the magnitude of this effect, but its reality is suggested by a tendency (with, however, some striking exceptions) in natural populations, of at least *D. pseudoobscura* and *D. persimilis*, for inversions giving long *simple* loops not both to be present in the same deme at

appreciable frequencies. "Long" is relative to the unknown frequencies of double crossovers; the longer the simple loop the less the likelihood of simultaneous abundance, by this hypothesis. Neither the results of single crossovers nor Wallace's triad effect (1953) would lead to this situation, which may be presented elsewhere in more detail.

Part of the preceding model may be questioned because MO apparently arose from CH in the cage (about 20 other cages started from the same stocks do not show it), and therefore should have CH genes. But there is no reason to doubt the lack of normal genetic heterogeneity in the 12 stocks from which CH was drawn, and, in fact, the rise in the frequency of MO is itself suggestive of this; see also Levine (1955) and changes in relative adaptive values in the present series of cages. The hypothesis is that MO incorporated a combination of genes heterotic with the average CH. Less well adapted inversions would be less likely to be found, even though they are known to occur (Dobzhansky, 1956). Superiority of the excessively rare MO homozygote or an advantageous position effect from the inversion itself are not excluded, but without MO/CH overdominance they make the effects, other than the initial rise in the frequency of MO itself, more difficult to explain.

The origin of the often widespread inversions in natural populations of many Diptera may have occurred by a similar unique incorporation of an advantageous gene complex, which could evolve subsequently as a unit. The new inversion would be most likely to survive where its parent sequence was relatively uncommon, because of double crossing over. However, since selection against the recombinants may not always be great, this is not a necessary condition and could, in any event, be overcome by sufficient heterosis with any sequence in the population.

I am indebted to Th. Dobzhansky for many kindnesses, and to Joan Mishara, Richard Levins, and Louis Levine for maintaining the cage during the field seasons of 1958 and 1959.

SUMMARY

An inversion in the third chromosome of *Drosophila pseudoobscura* appeared spontaneously in a population cage. It increased in frequency for several generations to a maximum of five per cent and then declined to elimination. A model is offered for this and other phenomena, and its significance is discussed.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

AN UNSUCCESSFUL ATTEMPT TO REDUCE RECOMBINATION BY SELECTION

It is many years now since Detlefsen and Roberts (1921) found that the amount of recombination between two genes in *Drosophila melanogaster* could be reduced by appropriately selecting the offspring which were to give rise to the next generation. A similar experiment had been tried by Gowen (1919), but for only six generations and with little effect. Since then this obviously important aspect of recombination has been neglected. Parsons (1958) in a letter to this journal showed that he had been able to *increase* the amount of recombination between certain genes, but this type of selection cannot be so effective because it must lead eventually to an increase in the number of double crossovers. I therefore repeated the experiment, using genes on another chromosome, to see whether the striking reduction achieved by Detlefsen and Roberts in the amount of recombination could be reproduced.

Detlefsen and Roberts used the genes *w* and *m*; in the course of ten generations they reduced the recombination frequency in one line from 27 per cent to 2 per cent, and in another line from 29 per cent to 6 per cent in 29 generations. Apart from the smaller number of matings used, I followed Detlefsen and Roberts' technique closely; they had an average of 40 and 61 matings per alternate generation in the two lines, I an average of 24, using instead a stock of *cn vg* and a wild-type stock formed by haphazardly mixing *Florida 4* and *Oregon K* stocks about 20 generations previously. The mixed stock was used in the hope of increasing the amount of variability which would be available for selection.

In the experiment, each even-numbered generation was a mass-mating of non-crossover offspring from the selected pair in the previous generation. In the following list, the first number is the recombination percentage of the total population in each odd-numbered generation; the second number is the recombination percentage of the single pair in this generation which was selected to give rise to the next generation.

10.4–8.4; 9.3–1.7; 8.8–4.3; 7.1–2.3; 7.6–3.9; 10.6–6.3;
10.7–5.5; 8.6–5.9; 9.9–5.7; 11.1–6.3; 8.3–4.4; 7.7–?

Three matings with high recombination frequencies, 16.3 per cent, 12.0 per cent, and 12.9 per cent, were set aside at F17, but the frequencies amongst their offspring were 8.5 per cent, 8.4 per cent, and 10.1 per cent.

Though there was a good deal of variation within the successive generations, it proved impossible to reduce the overall frequency of recombination after 23 generations. The recombination frequency calculated from the grand total of all the generations is 9.3 per cent, and agrees closely with the generally accepted crossover value of 9.5 per cent.

The theoretical aspects of this problem have been amply discussed by Gowen (1919), Detlefsen and Roberts (1921), and by Detlefsen and Clemente (1923). There is little point in speculating here on the reasons for success or failure, except to remark that Detlefsen and Roberts used sex-linked genes.

I am most grateful to Dr. P. M. Sheppard for suggesting this problem, and for his interest in the outcome.

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ON THE FUNCTIONS AND CAUSES OF SEXUAL DIMORPHISM IN BREEDING PLUMAGE CHARACTERS OF NORTH AMERICAN SPECIES OF WARBLERS AND ORIOLES

In the course of a general review, soon to be published elsewhere, of the functions and causes of sexual dimorphism in the coloration of the bird plumage, attention was directed to certain North American species of wood warblers (Parulidae) and orioles (Icteridae). For these species it has generally been assumed (see, for example, Beecher, 1950; Skutch, 1957) that the dimorphism is a result of selection in females for less conspicuous appearance. In related species (congeneric or confamilial) endemic to the lower latitudes of Middle America, however, conspecific males and females are often identical in plumage appearance, even though there is no evidence that adult female mortality is less in tropical than in temperate latitudes.

Without excluding the possibility that selection for avoidance of predation is one factor contributing to the "dulling" of the plumage of the female sex in these North American species, I would like to present another hypothesis—one that relates dimorphism of plumage characters to behavioral adaptations (for example, for alleviation of inter-sex hostility, see Moynihan, 1960) associated with conspecific mate location and selection (pair-formation), maintenance of pair-bond, and length of the prenesting "engagement" period. To test this, sexual dimorphism in the warbler genus Dendroica and the oriole genus Icterus was reviewed, and is here cited as demonstrating the following model. The reader is referred to standard ornithological publications for documentation (see, for example, Skutch, 1957: 277–278) of the south-to-north, monomorphic-to-dimorphic trends of plumage coloration in these genera. In the following discussion, the term "pair-bonding behavior" is used to denote displays associated with both the formation and the maintenance of the pair-bond.

All members of these two genera are pair-forming, territory-defending species, at least during the breeding season. Middle American species are usually monomorphic in plumage appearance, sedentary, and tend, as far as known, to maintain an annual or permanent pair-bond. The plumages of these species of the lower latitudes are usually brightly colored, and, in adults, are constant throughout the year. The North American species, on the other hand, are usually migratory, establish only seasonal pair-bonds, and are mostly dimorphic in plumage characters. In these dimorphic species, the males have a conspicuous plumage which, in some species, is replaced by an inconspicuous plumage during the non-breeding season (see Hamilton and Barth, 1961). Skutch's data (1957) on North American species of warblers wintering in Central America indicate that they occur there for six months or more of the year. Allowing several months for the time required for two annual migratory flights, the warblers breeding in North America probably spend two or three months in pair-bond. The same seems true for North American orioles (R. H. Barth, Jr., personal communication).

As is true of many other passerine species which form only a seasonal pair-bond, the males of North American warblers and orioles arrive at the breeding habitat before the females, establish territories, and defend them from conspecific males. If we assume that (1) these northern migratory species are descended (see Mayr, 1946) from monomorphic, conspicuously colored relatives resident in lower latitudes, then we may infer that the sexual dimorphism in the species breeding in higher latitudes is partly a result of the "loss" or "dulling" of certain plumage characters in the female sex. Now, if we assume that (2) a female—wearing the same plumage characters that elicit hostile behavior between conspecific males—arriving at an unmated conspecific male's territory would be the recipient of hostility from that male, then we may infer that the pair-bonding process will be less efficient or require a longer period of time than if she had not (or had in modified form) those certain plumage characters.

Could it be that the loss of certain plumage characters by the female of these North American species represents one of several ways whereby selection can operate for resolving the problem of reducing hostility at the time of pair-bonding? It has occurred to me that sexual differences in plumage characters might operate for rapid sex-recognition and alleviation in part of hostility between potential mates. This might also permit (?) a relatively more rapid breeding process in an environment less constant than that to which these species are historically adapted.

A corollary of the above hypothesis is that a relatively longer period of time for pair-bonding and nesting behavior is associated in monomorphic bird groups with selection for the development of alternate adaptations for sex recognition and reduction of inter-sex hostility—*viz.*, ritualization, appeasement displays, etc. Those familiar with the extended and mutual pair-bonding displays of sexually monomorphic groups such as the gulls (Laridae; see Tinbergen, 1959) or the herons (Ardeidae; see Meyerriecks, 1960) will realize the plausibility of these comments.

To my way of thinking, the above concept is not in conflict either with the concept that species-specific plumage characters are due to interspecific selection pressures, or with the concept that conspicuous plumage characters are due to sexually differential intraspecific selection pressures stemming from recognition problems associated with, say, mate location. All three concepts should be considered complementary, and specific plumage characters may well serve for any one or more of the three functions here cited.

These considerations are speculative and theoretical, but they can be tested. There is a need for further investigation of the possibility that sexual differences in plumage pattern and/or coloration may operate for (among other functions) rapid sex-recognition and partial alleviation of inter-sex hostility during the biologically important pair-bonding period. The fact that the presence or absence of hostility (this is necessarily an over-simplification of the matter) is communicated between birds by dis-

plays which consist of plumage and/or motion and/or vocal releaser signals indicates a potential array of adaptive solutions to this problem. It would be interesting to relate these conclusions to Tinbergen's concept (1959) of behavioral adaptations for "reducing the distance" between individuals.

Finally, although this report is based on adaptive trends manifested by members of two New World genera, similar comments may be applicable to many other dimorphic species burdened with the ethological problem of annually establishing and breaking the pair-bond—to cite only a few: the North American member(s) of Turdus, Piranga, and Dolichonyx; the buntings of the Emberiza-Plectrophenax complex; and the Palearctic species of Oriolus and Pyrrhula (*viz.*, off-season deserters of the breeding territory).

In developing this hypothesis, I have benefited from discussions with Drs. Ernst Mayr, Andrew J. Meyerriecks, Robert H. Barth, Jr., W. John Smith, and Robert H. MacArthur.

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January 7, 1961

THE EFFECT OF NEUTRON IRRADIATION ON THE FERTILITY OF *TRIBOLIUM CONFUSUM*

The precision with which many biological attributes of the flour beetles, *Tribolium confusum* and *castaneum*, can be measured make them eminently suitable for radiobiological research, and the growing interest in the genetics of Tribolium will probably enhance this suitability. However, the only extensive experiments reported to date have been with x-irradiation (Park, 1958; Ducoff, 1960), and since radiations of different ion densities are known to have different biological effects (for example, see Alexander, 1958), an investigation of the effects of fast neutrons on *Tribolium confusum* was initiated. The results reported here are of a preliminary nature, but more extensive experiments are now in progress.

The neutrons, obtained from five Pu-Be sources, each with an activity of one curie, have an energy distribution consisting of 55 per cent with 0–5 Mev., 38 per cent with 5–9 Mev., and seven per cent with 9–10 Mev. The sources were arranged in a circle forming a central well which was fitted with a removable lucite chamber containing the biological material. A neutron flux of about 1.3 to 1.6×10^5 n/cm^2/sec. and gamma irradiation at a rate of 1.2 to 1.5 r/hr were calculated for the position of the chamber.

Five adult males about ten days old were placed in the lucite chamber along with three grams of the whole wheat flour-brewer's yeast medium commonly used for culturing Tribolium. The chamber was then lowered into position among the neutron sources and allowed to remain there for 96 hours. The neutron dose received by the males was estimated to be about 500×10^6 n/cm^2 or, according to the conversion tables of the NBS Handbook 63 (1957), about 254 rads. A control, consisting of five adult males of the same age in an identical chamber and supplied with flour-yeast medium was kept in the same room during this period.

All the males in both control and experimental groups were then placed in separate vials, each containing ten grams of medium and a virgin female at least five days old. After two days, the males were transferred to different females for another two days and this process was continued until each male had been mated six times over a twelve day period. Each female was permitted to deposit eggs in the vial for five days after the male was removed and then the female was discarded. About five to six weeks later, the total number of adult offspring in each vial was determined. The data and analysis of variance are given in table 1. The absence of offspring from male 7 in period 5, probably due to sterility of the female, made it necessary to include a correction for disproportionate subclass numbers. The difference in fertility between the irradiated and control males is clearly significant ($F = 36.2$, $n_1 = 1$, $n_2 = 47$), but there seems to be no significant change in fertility during the six observation periods. Since the controls were expected to remain steady, the experimental data were analyzed separately, but no significant change in fertility over the six periods was found ($F = 2.4$, $n_1 = 5$, $n_2 = 24$).

TABLE 1

The number of offspring produced by each male during each two day period. The symbol ** denotes significance at the one per cent level.

Treatment	Period					
	1	2	3	4	5	6
Irradiated ♂♂						
1	71	58	67	55	60	64
2	58	53	43	55	92	67
3	72	65	63	49	70	45
4	65	67	53	48	70	34
5	75	66	24	50	52	67
Mean	68.2	61.8	50.0	51.4	68.8	55.4
Control ♂♂						
6C	80	57	87	62	92	65
7C	77	74	54	76	0	84
8C	85	70	78	76	66	80
9C	74	89	71	92	91	79
10C	76	63	86	81	74	90
Mean	78.4	70.6	75.2	77.4	80.7	79.6

	d.f.	s.s.	m.s.	F
Treatments	1	4684	4684	36.2**
Periods	5	1191	238	1.8
Interaction	5	828	106	1.3
Individual	47	6077	129	

The male germ cells of *Drosophila virilis* in various stages of development have been shown to differ in their sensitivity to neutrons (Alexander, 1958). It seemed possible, therefore, that a similar situation would be found in *Tribolium confusum* and manifest itself as a variation in fertility from one period to the next. While our data do not indicate the presence of differential sensitivity in *Tribolium confusum*, it would be unwise on the basis of this preliminary study to conclude that it does not exist. Experiments now in progress utilizing x-rays as well as neutrons, should provide more conclusive information concerning the effects of radiation on the male germ cells of *Tribolium confusum*.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Mrs. Nancy Peer for her assistance. This work was supported by research grants G-8888 from the National Science Foundation and RG-5836(R 1) from the U. S. Public Health Service.

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December 20, 1960

COMMENT ON DR. F. HUTCHINSON'S PAPER AND REPLY TO OUR LETTER

In his reply to our letter (Stein and Laskowski, 1960; Hutchinson, 1960) Dr. Hutchinson pointed out that our remarks concerning the same magnitude of order of x-ray doses necessary for the induction of recessive lethal mutations and for DNA inactivation are only consistent if the main inactivation effect in yeasts is caused by recessive lethal mutations. Here seems to be a misunderstanding about the suppositions of our quantitative analysis. Besides the statement mentioned above, the prevailing of recessive lethal mutations in the inactivation of haploid Saccharomyces strains is a further result and not a supposition of this analysis. Furthermore, our analysis shows that in diploid and polyploid cells inactivation is caused predominantly by dominant lethal mutations (Stein and Laskowski, 1960). The disclosure of this difference in the main inactivation mechanisms between haploid cells and cells of higher ploidy is in accordance with the findings of Mortimer and the statement of Magni. We therefore should like to reiterate our opinion that at least in haploid Saccharomyces strains the x-ray inactivation effect is consistent with the radiation sensitivity of macromolecules, either DNA or enzymes.

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